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BEARING ON HEREDITY AND VARIATION

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John Billings

JOHN BELLING

JOHN BELLING, was born in Aldershot, England, October 7, 1866. He was educated in private and public schools, spending two years at the University of Birmingham and receiving the degree of B.Sc. at the University of London in 1894. After teaching in the lower schools he was research lecturer at the HORTICULTURAL COLLEGE AT SWANLEY, 1895–98, and for the succeeding three years, teacher of science at Wells, England, and Llandidloes, Wales.

In 1901 he was sent to the Island of St. Christopher in the West Indian service of the IMPERIAL DEPARTMENT OF AGRICULTURE. He remained in that service until 1907 when he visited Florida and joined the Staff of the STATE AGRICULTURAL EXPERIMENT STATION. After one year he became Assistant Botanist in the Experiment Station and Editor of the station publications and remained in this post eight years.

BELLING'S first scientific contribution dealt with the cytology of polyembryony in the mango, *Magnifera Indica*, published in 1908. Two years later he began his classical studies on the genetics of certain interspecific hybrids in the genus *Stizolobium*, which led to several discoveries of fundamental importance, particularly on the mode of inheritance of semisterility, which later found its cytogenetic explanation in his hypothesis of segmental interchange between chromosomes.

During the next five years BELLING was not officially connected with any institution but, in the latter part of this period, he became associated with DR. A. F. BLAKESLEE at the Station for Experimental Evolution of the CARNEGIE INSTITUTION OF WASHINGTON at Cold Spring Harbor, N. Y. From the first he devoted himself to the cytological aspects of the *Datura* investigations and in 1924 he was appointed Cytologist in the CARNEGIE INSTITUTION which position he held until his death FEBRUARY 28, 1933.

It was at Cold Spring Harbor that he began his intensive study of chromosomes and their behavior which was continued throughout the rest of his life and which entitle him to be known as pioneer in cell mechanics. For BELLING was the first, at least among botanists, to utilize both the genetic and the cytologic viewpoints in attempting to analyze the results of breeding experiments. He had unusual mathematical ability and a grasp of chemistry and physics which he applied in perfecting the technic necessary to success. His thorough understanding of optics is shown by his book on the microscope. His contributions to stain technology are of first rate importance. He developed the aceto-carmine method of staining by the addition of iron which made it possible to observe the chromosomes in stages of meiosis with greater facility and accuracy.

BELLING studied these phenomena in several plant species including *Canna*, *Cypripedium*, *Hemerocallis*, *Hyacinthus*, *Uvularia* and *Tulipa;* and in the *Datura* investigations which were one of his chief interests during this period he gave the final answer to many problems which the breeding behavior had

raised and predicted genetic results from his observations of the chromosomes. His basal contribution, from which other hypotheses such as segmental interchange were developed, was his idea that the arrangement of chromosomes in pairs in the first meiotic division is due to the organization within the individual members of a pair such that like ends are attached in this stage. By identifying specific ends of chromosomes in groups of more than two he discovered evidence for the specificity of the extra chromosome in trisomics at a time when it was believed by some investigators that the extra chromosome is a characteristic rather than a cause of trisomic types and that it is a matter of no consequence which chromosome is extra. His influence in changing cytogenetic concepts can be further realized when it is remembered that until very recently it was held that the attachment of chromosomes into circles, so common in some species of *Oenothera* and so frequent in *Pisum* and *Zea*, is due to a general affinity between maternal and paternal chromosomes or that circle formation is a genetic character inherited like any other genetic character due to a gene.

As he had suffered from ill health for several years, in 1927 it was arranged that BELLING should go to California. At that time The Division of Plant Biology of the CARNEGIE INSTITUTION OF WASHINGTON AT STANFORD UNIVERSITY had not been established; so accommodations were provided by the Division of Genetics of the UNIVERSITY OF CALIFORNIA. At Berkeley he continued his work for the remainder of his life, holding the position of Research Associate in Genetics in the University without salary and continuing as Cytologist in the CARNEGIE INSTITUTION. Under these conditions he was able to renew his investigations with fresh vigor. Many different projects were planned and a surprising number were brought to completion within a comparatively short time. His first major undertaking was the study of the finer structure of the chromosomes. After much effort he was able to estimate the number of chromomeres in the pollen mother-cells of a lily and he showed that this number, about 2,200 pairs, agrees with expectations from genetic evidence, since the orientation and behavior of the chromomeres during cell division are such as would be expected if the chromomeres contain the genes. Then, by a special technic he demonstrated the existence of an ultra-microscopic particle near the center of each chromomere which he believed could be identified as the gene. Thus he anticipated, by his painstaking and critical research on normal chromosomes in plants, the important discoveries of structure and organization of chromosomes which have come recently from investigations on the salivary glands of certain insects. It was in connection with these studies that he perfected the iron-brazilin method of staining which he found more satisfactory for some materials and for certain purposes than the iron-aceto-carmine method.

During the last three years of his life, BELLING concentrated his efforts with unabated enthusiasm and increasing clarity of penetration on a problem of major importance to genetics, viz., the nature of the mechanism of crossing over or interchange of segments between chromosomes. Two days before his death in 1933 he brought to completion the manuscript of his final paper in a series of valuable contributions on this complex problem. This paper (*Genetics*,

18: 388–413, 1933) presents the evidence for his hypothesis and its application to cogent genetic data. In order to convey more clearly his conception of the mechanism of crossing over and other chromosomal rearrangements, he prepared a series of models which were exhibited by the Carnegie Institution in Washington and New Orleans in 1931–32 and some of them were shown at the Sixth International Congress of Genetics in 1932.

It was only by combining unusual skill in technic and observation, rare ability in formulating hypotheses with a strong factual foundation, and a dogged determination to succeed in spite of all obstacles, including his own physical frailty, that John Belling was able to win the place of honor he deserves in the early development of the new science of cytogenetics. Always timid and retiring he preferred to work in obscurity, and indeed the only public recognition he received was the honorary degree of Sc.D., awarded by the University of Maine in 1922. Yet to a few who came to know him intimately, either by personal contact or through his published poems, Belling revealed an unsuspected philosophical insight and a truly artistic temperament. Rather late in life he married Dr. Hannah Sewall, a woman of culture and refinement. The short span, some five or six years, before her death seems to have been a period of great spiritual uplift for him. Some of his poems, particularly the "Elegy," reveal how deeply the artistic side of his nature was influenced by this all too brief companionship.

In his own laboratory Belling was always at home and ready for visitors. To students especially he gave a cordial welcome and a sympathetic hearing. Impatient of mere conjecture without factual basis, he was extremely careful in his own writing and he set a standard of excellence in this, as in all his work, which may well serve as a guide and inspiration for others.

This biographical sketch was prepared by Professor E. B. Babcock in collaboration with Dr. A. F. Blakeslee who a so furnished the photograph for the frontispiece.

GENETICS
1916-1936

WITH this issue GENETICS enters its twenty-first year. From its inception it has been conducted by the Editorial Board of ten members which founded it, and by two editors who have served it for periods of ten years each. To the first editor Professor George H. Shull and to the retiring editor Doctor Donald F. Jones, the Board and all students of Genetics owe a great debt of gratitude for their effective and unselfish services.

GENETICS is owned by its Editorial Board, a self perpetuating body which participates actively in publication and management of the Journal. The original board has recently enlarged itself by the election of five new members, Leon J. Cole, John W. Gowen, Alfred H. Sturtevant, Sewall Wright, and L. C. Dunn. The last named assumes the editorship with this issue. On the occasion of its twenty-first birthday GENETICS welcomes the new members to its Editorial Board.

INHERITANCE AS IT AFFECTS SURVIVAL OF RATS FED A DIET DEFICIENT IN VITAMIN D

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Received August 17, 1935

THOSE familiar with experiments on diets realize that within a group of animals given the same food in like quantity, the reactions of individuals may be quite diverse. On another diet a similar group may show similar individuality but the curve of such variation may be distinctly separate from that of the first group. Experimentation in nutrition has attempted to eliminate individual variation in the animals and to emphasize the differences between given diets. In the light of the objectives sought, this attitude is commendable. Individual variation within experiments, however, seems to have a significance not to be overlooked. Individuality as a cause of variability has the same primary importance with respect to the characteristic pathology which animals develop under experimental dietary conditions as under those of nature. The food requirements of even closely related genera may often be quite distinct. From the point of view of evolution, this seems significant since the causes of such differences in food requirements are fundamental to the correlation between the spread of animals and their subsequent development.

For the study of diverse individual variations which animals may show toward a given diet, vitamin D was chosen as the major variable in our experiments. This nutrient principle is apparently a single chemical entity necessary for normal body development and maintenance of many vertebrates. The symptoms which develop in the absence of it depend somewhat upon the age of the animal. A lack of it in the young, when bones are developing actively, may lead to rickets and osteoporosis, due to improper deposition or actual extraction of calcium from growing skeletal structures. Growth is impaired or stopped; teeth are poorly calcified and often pitted. A deficiency at puberty may be correlated with crippling and painful changes which often lead to difficulties in reproduction. Such conditions are more striking in females than in males. In the pregnant mother, excessive fetal demands for calcium and phosphorus may induce symptoms of osteomalacia. Later, the nursing mother often shows somewhat the same pathological conditions due to similar demands in the production of milk. In middle-life, osteomalacia sometimes develops if the diet is sufficiently inferior, although the changes tend to appear less rapidly than in the young. Birds show particularly severe symptoms in the form of leg-weakness which may be rapidly fatal in the young chick.

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Tetany often follows long-continued deprivation in some cases. Deficiency is also accompanied by reduction and degeneration of the spleen, deposition of calcium in the walls of arteries, a reduction of blood-clotting time, hemorrhage and striking change in the parathyroids. An increase in the size and number of epithelial cells of the parathyroids is followed by shrinkage and regression of the cell cords and, in some cases, a hyperplasia of the stroma. Marked keratinization then takes place. The antirachitic principle may be supplied through food or through exposure of the body to direct sunlight. In the latter case, the color of the exposed skin materially influences the amount of sunlight absorbed and hence its antirachitic effect (1).

The question which will be studied in this paper is the effect of inheritance on the utilization of vitamin D. The criterion for measuring the effect is the variation in the length of time that the animals survive when fed a diet deficient in the vitamin.

MATERIAL

Genetic research has shown that a population of animals bred at random, or nearly so, generally contains different inherited potentialities. Two individuals, though they be carefully selected are apt to deviate in their reaction to any given variable even if the experiment is well controlled. It is evident that this random differentiation would vitiate the analysis we propose. The aim is to keep the full spread of inherited differences of the initial population, but to segregate it into lines each of which has a distinctly reduced inherited variation. Close inbreeding of the brother and sister type furnishes a means by which this end may be accomplished. If from a population of rats, we select a dozen pairs, we have a sample of the inherited potentialities of the entire group. Any given pair will be likely to contain but a limited part of the variation in the total spread of inheritance. The progeny of such a pair will tend to have only a portion of the inherited variation which their parents possessed, although considered collectively, the whole litter, if large, might well contain all of it. Each generation, so long as the inheritance of the line comes through a single pair, will consequently become more and more alike, until a time is reached when the progeny within a given generation are so closely similar that they can be considered essentially alike in respect to inheritance. Any variation which such a population shows may therefore be attributed to environment. Those familiar with the effects of such factors as linkage and balanced lethals will realize that even here difficulties may enter. However, the same procedure in inbreeding each of the sample lines originally selected at random will result in uniformity within each of the lines, but between the lines, if the selection is fortunate, the full inherited

variation of the original random population will remain. The differences between such lines may be used to measure the influence which inherited variation may exert on the particular variable studied.

The experiment described here was fortunate in having available to it 16 lines so distinctly differentiated in their inheritance. We are indebted to Dr. M. R. CURTIS of the INSTITUTE FOR CANCER RESEARCH at COLUMBIA UNIVERSITY, New York, for the animals which we used to start each of our lines, and for the data from which it has been possible to obtain the survivorship of these lines when fed our complete diet (CURTIS and BULLOCK 1923; CURTIS, DUNNING and BULLOCK 1933). The sixteen strains were originally from the stock rats of four different dealers and from one European laboratory strain. When the selected pregnant females were transferred to Rockefeller Institute at Princeton, New Jersey, the different lines had been bred brother by sister for 6 to 9 generations. Three to 5 generations of further inbreeding intervened before the animals were used in the experiment. Care was taken to avoid the introduction of transmissible diseases and parasites into these new lines. Before transfer, the pregnant females were examined thoroughly and dipped to eliminate any external parasites. After transfer, they were separated from each other by solitary isolation in different laboratories. Here the young were born and at weaning time the mothers were autopsied. Lungs were examined especially for pneumonia, feces for paratyphoid bacilli and the eggs of nematode worms. All females abnormal in any of these particulars were eliminated along with their litters. The remaining litters were then bred and the process repeated in like manner. Finally the stock was free of paratyphoids, nematodes, skin parasites and pneumonia, for a time at least. Unfortunately we missed the middle ear focus of the actinoides organism and as a result the pneumonia which it favored subsequently appeared in our stock. We could not find that its incidence had any distinct relation to the deaths in different lines whether they were subjected to the vitamin D deficient diet or to the normal one. That the disease is capable of control by the measures taken is indicated by the fact that it has been eliminated in one strain of our stock (NELSON and GOWEN 1930).

The untreated rats were maintained on the following stock ration. Bread, whole milk, fresh vegetable and yellow corn were fed on Monday, Wednesday and Friday; bread, milk and cereal on Tuesday and Thursday; bread, milk, cereal and yellow corn on Saturday. The cereal was composed of equal parts of cracked whole wheat, rolled oats and yellow cornmeal mixed with milk and cooked for 20 minutes. A special rat biscuit and water were before the animals at all times. Meat was fed once a week. Such a diet was chosen for the untreated rats because of its wide variety of foodstuffs and its ability to maintain the animals' condition.

The choice of a diet for the test animals presented many difficulties. Two diets low in vitamin D were in common use; that of McCollum, 3143 (1922), and that of Steenbock and Black, 2965 (1925). Both rather readily produce the vitamin D deficient picture in rats fed on them. The calcium-phosphorus ratio is unbalanced in both, the calcium being high and the phosphorus low. Furthermore, both diets are believed to be deficient in vitamin B_2, and also in certain minerals and iodin. Either diet, however, has in its favor the real advantage of having been widely used in other feeding experiments. Since comparability with the experiments of others seemed desirable, it was decided to use the 2965 diet in preference to any new ration which we might devise. The proportions of this diet were 76 percent of ground yellow corn especially selected for its brightness of color, 20 percent of wheat gluten, 1 percent of sodium chloride and 3 percent of calcium carbonate. Distilled water was continuously before the animals.

The experiment was conducted in the following manner. Rats to be fed the vitamin D deficient diet were kept out of contact with direct sunlight. Females were isolated from the time that pregnancy was noted and the resulting litters were kept separate from other rats. The litters were weaned 3 weeks after birth and from then until 46 days of age, when the vitamin D deficient diet was started, they were fed on the regular stock ration. At 46 days of age, the young were put into individual screen-bottom cages 9 inches square and held $\frac{3}{4}$ of an inch above the shelf and away from direct sunlight. Food was always present in the cages. Its daily consumption was reckoned on the difference between the weight put into the cage on one day and that remaining the next. It is evident that animals could obtain some of the metal elements from the wire of their cages; other than that and their diet, it is believed that they received nothing. In the daily routine procedure, all cages were examined for feces which might have caught on the wire bottom, and shelves under the cages were cleaned. The animals were weighed once each week.

Data pertinent to the problem included litter size, initial weight at 46 days of age, maximum weight, age when maximum weight was attained, time of death and weight at death. From these items the rate of gain and absolute gain from initial to maximum weight may be calculated. The subsequent loss in weight and rate of loss until death may also be determined. The food intake was found to rise somewhat at first, then to remain quite constant until the week of death when the records show some drop. In view of the fact that differences in litter size and in weight are regarded as in part hereditable and since the aim of the experiment was to test inheritance, no adjustment in litter size and weight was made. Obviously such a procedure would have overthrown some of the objectives of the

experiment. This problem is approached, however, by what is possibly a better technique in a later section of the paper.

Originally it was intended to revive some of the long-time survivors of the diet deficient in vitamin D by giving them a normal diet and ultraviolet light. The plan was not carried far because rats, although revived in many respects by such treatment, were found to be sterile.

It is clear that although the experiment is directed at the effect of inheritance on a deficient supply of vitamin D, other more or less significant nutrients have been reduced. We should, of course, like to say that the results obtained relate entirely to vitamin D and the effects of inheritance in making this element more or less essential for the metabolism of one strain but not indispensable to a strain of different genetic constitution. This, it seems, we can not do completely, if for no reason other than our present ignorance of many essentials of nutrition. All the experiment can hope to indicate is that given a diet with certain deficiencies, vitamin D important among them, the susceptibility of different groups varied, and that a portion of this variation was attributable to inheritance. In referring to the subsequent results the term "vitamin D deficient diet" is intended to convey the broader definition. Considered from the point of view of evolution, such variations may indicate the adaptability of certain races to border zones deficient in particular environmental factors and in DAVENPORT'S sense may fit races for invasion into what would otherwise be inhospitable regions.

The present paper directs attention to the variation in length of life of genetically different strains of rats fed the same vitamin D deficient diet. We may begin our study, however, by an analysis of the variation curves of the lives of all of the rats taken together. Through the courtesy of Dr. M. R. CURTIS in supplying the data, it has also been possible, for the benefit of the reader, to place on the charts life curves of rats of the same strains when fed a completely adequate diet. A total of 4981 male and 7607 female completed lives are represented in this material.

LIFE CURVES OF RATS ON THE VITAMIN D DEFICIENT DIET

Data from all lines were combined into a life curve representing the effects of the vitamin D deficient diet on the whole population. The total data compared with those for the human life tables are rather pitifully small, for collecting material under the conditions and requirements of the experiment is slow work. But surprisingly smooth curves result. There are also the real advantages that the data were collected with a definite purpose in view and that all the entering variables were recorded at the time of occurrence and not at death.

SEX DIFFERENCES IN THE REACTION TO THE VITAMIN D DEFICIENT DIET

Sex, of course, is an inherited character, being dependent on the chromosome distribution, although the conditions bringing it about have thus far defied exact analysis. Since a sex difference is manifest in the data, the original curve is divided into 2 curves, 1 for each sex. The tabulation represents survivors against duration of life, duration of life being measured as age in days divided by 30. The males in table 1 show a somewhat greater duration of life than the females.

TABLE 1

Survivorship curves of rats on vitamin D deficient diets.

AGE DAYS/30	NUMBERS WHICH SURVIVED	
	MALES	FEMALES
2	170	167
3	162	154
4	141	139
5	108	104
6	78	73
7	60	50
8	44	29
9	32	15
10	25	8
11	16	2
12	11	1
13	6	
14	5	
15	4	
16	1	

Figure 1 gives the graph of these results. If we test the significance of differences between the sexes by comparing the specific death rates, we find that the χ^2 value for 10 classes is equal to 14.7; the P value is slightly less than 0.1 (ages of 10 and above are grouped into one class). We should have to conclude that sufficient data were not at hand to prove that there was a distinct difference between the sexes. But the fact that a sex difference is manifest in our data in the direction of a more severe effect of the vitamin D deficiency on females than males is in line with observation on the human in puberty and after-life and in my own unpublished results on the domestic fowl. A similar effect has been noted by BILLS and others (1931) in line test studies on rats, the males showing a slightly greater rate of healing than the females.

Sex differences in reactions to specific agents of disease and to other agents are becoming rather well known. WRIGHT and LEWIS (1921) have

shown such differences in the resistance of guinea pigs to tuberculosis. In their experiments, as in ours on vitamin deficiency, the females died more rapidly than the males. In mice the writer's experiments are showing a reversal of this effect: exposure of a population to the effects of ricin poison causes the males to die at distinctly greater rates than the females (GOWEN and SCHOTT 1933). In rat and mouse typhoid, and mouse liver disease (*B piliformis*), however, the sexes react alike (IRWIN 1929; SCHOTT 1932; WEBSTER 1933; GOWEN and SCHOTT 1933).

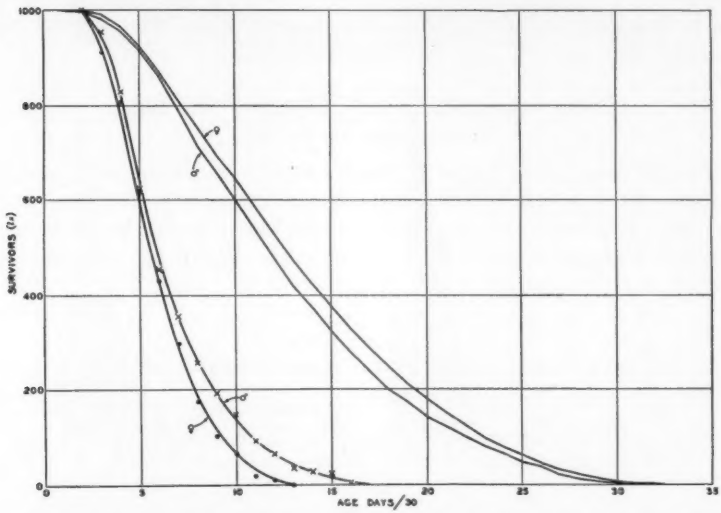

FIGURE 1.—Curves showing the survivorship plotted against age for rats on vitamin D deficient diets, left-hand curves, and like rats on a normal diet (based on CURTIS' data), right-hand curves.

A comparison of the left- and right-hand curves in figure 1 shows that rats on a diet believed to be adequate have a distinctly longer life-span than those on the deficient diet. In the former group, 11–12 months elapses before 50 percent of the population dies, in the latter only 5–6 months intervenes. After this point, the survivors of the deficient diet begin to die off more sharply than the others and the form of the curve therefore changes. This type of curve is also found when Drosophila are subjected to starvation. Apparently when one major cause of death overshadows all others the curves tend to have an abrupt, relatively limited cycle. This suggests the destruction of a reserve substance at a given rate. The fact that the rats on the vitamin D deficient diet lived but one half as long as those on the complete diet is sufficient evidence to emphasize the importance to

life of the deficiencies observed in this diet. These differences are without regard to the inheritance since the inheritance within two groups is quite similar.

The sex differences in rats on the normal diet are well marked, no question of their statistical significance being possible. They are, however, in a direction opposite that of rats on the vitamin D deficient diet, that is, the males die sooner on the normal diet than the females. Such a difference between the rats on the two diets seems significant, for while it is not possible to assert that the male and female rats are strictly not identical, the probability that the females would live longer than the males, as they do under the normal diet, becomes rather small, about 1 in 100.

CHARACTERISTICS OF THE CURVE SHOWING THE FREQUENCY OF DEATH

The constants of the frequency curves of the population, variable in its heredity, have special interest when they are compared with those of the like population when the variation brought about by the heterogeneous heredity is removed. The frequencies of death against age are shown in table 2 for the population lacking vitamin D.

TABLE 2

Frequency of deaths of rats on a vitamin D deficient diet.

AGE DAYS/30	NUMBERS WHICH DIE	
	MALES	FEMALES
2	8	13
3	21	15
4	33	35
5	30	31
6	18	23
7	16	21
8	12	14
9	7	7
10	9	6
11	5	1
12	5	1
13	1	
14	1	
15	3	
16	1	

Figure 2 shows the frequency polygons, in which percentages of death are plotted against age for rats on normal and vitamin D deficient diets. The two sexes are represented separately. The striking feature of these diagrams is that animals on the vitamin D deficient diet reach a much

higher total death rate per unit of time than those on the normal diet. For purposes of comparison, the constants of PEARSON'S frequency curves are useful in clarifying these differences. These constants are found in table 3.

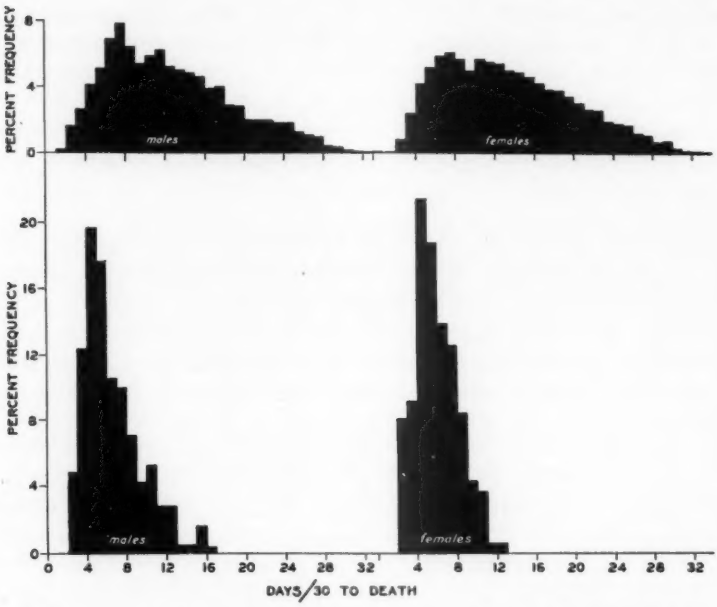

FIGURE 2.—Frequency diagrams for percentages (upper figure) of death plotted against age for rats on normal diets (derived from CURTIS' data), and on vitamin D deficient diets (lower figure).

TABLE 3

Frequency constants for deaths of rats plotted against age, normal and vitamin D deficient diets

	VITAMIN D DEFICIENT DIET		NORMAL DIET (CURTIS DATA)	
	MALES	FEMALES	MALES	FEMALES
Mean (days)	197.3 ±4.6	178.3 ±3.3	379.4±1.8	403.6± 1.5
Standard deviation	89.0 ±3.3	63.4 ±2.3	189.9±1.3	198.1± 1.1
Coefficient of variation	45.2	35.6	50.1	49.0
μ_2	8.80	4.46	40.13	43.6
μ_3	28.85	4.77	159.40	151.3
μ_4	299.22	56.13	4406.85	4840.4
β_1	1.22± .27	0.26± .11	0.39± .02	0.28± .01
β_2	3.86± .48	2.82± .25	2.74± .04	2.55± .02
Skewness	1.27± .38	0.41± .10	0.77± .04	0.70± .03
Type of curve	I	I	I	I

The constants of table 3 show that the frequency distribution of the deaths of rats on the vitamin D deficient diet has a distinct positive skewness. The curve prescribed for these distributions is PEARSON'S Type I.

If we compare the constants derived for animals on the vitamin D deficient diet with those for a similar group on the normal diet (CURTIS' data), we note that while the average duration of life in the latter animals is not quite twice as great as that of the former, the standard deviation is more than twice as large. The coefficient of variation consequently shows a relatively greater variation in the deaths of rats on the normal than on the vitamin D deficient diet. The skewness of both curves is within the same range. The constants of both sets of observations lead to Type I curves.

CHARACTERISTICS OF THE FREQUENCY DISTRIBUTIONS OF DEATHS OF THE SEXES WHEN EACH GENETICALLY SEPARATE GROUP IS CENTERED ON ITS MEAN

We may turn to the question, what are the characteristics of the death curves when the hereditable variation in the susceptibility of the animals is removed? Inbreeding tends to purify a race in the sense of making one animal genetically like the other. The strains of rats used in these experiments had all been inbred nine or more generations by brother and sister matings. They should, therefore, be quite pure from the point of view of inheritance. The variation which remains within each strain may be looked upon as that due to external environmental conditions. Any differences between the strains, on the other hand, may be assigned to differences in the genetic constitution of the strains. By centering the variability curve of each strain on its mean, we eliminate the variation due to heredity and are in a position to examine the variation due to environment. The constants of the distributions of deaths for male and female rats on the vitamin D deficient diet are seen in table 4.

TABLE 4

Constants of the distributions of deaths for rats on vitamin D deficient diet when each genetically separate group is centered on its mean.

	MALES	FEMALES
Mean	−0.0 days	−0.2 days
Standard deviation	56.1±2.0 days	42.16 ±.6 days
μ_2	3.49 days	2.02 days
μ_3	1.98 days	0.36 days
μ_4	52.85 days	13.39 days
β_1	0.092± .186 days	0.015± .010 days
β_2	4.187±2.43 days	3.036± .365 days
Skewness %	0.096± .083 days	0.061± .064 days

When each genetically separate group is centered on its mean, the standard deviations have become markedly reduced. This shows that the strain differences played a real part in the resistance of the population to the lack of vitamin D. The frequency distributions have become more symmetrical, the Beta constants do not differ significantly from the values of the normal curve $\beta_1 = 0$ $\beta_2 = 3$. The skewness of both the male and female groups has been eliminated with the removal of the major inheritance differences. The frequency curves are now essentially symmetrical.

The constants indicate that when the heterogeneity introduced by differences in inheritance is removed, Gaussian curves will approximate the survival curve of the rats. This fact has particular significance to studies of the potencies and effects of vitamin D preparations since the interpretation to be placed on such assays so often depends on the statistical significance to be attached to them. The approximation of these distributions to normal curves has a further interest to other studies of physiological resistance, that is, the effect of inheritance on disease resistance, since it is often necessary to determine the perimeters of the curves for the resistance of the host to the given disease-provoking entity before an analysis of the inheritance of disease resistance is possible.

EVIDENCE FOR THE EFFECTS OF INHERITANCE ON RESISTANCE TO VITAMIN D DEFICIENCIES

The mean durations of life of the different lines of rats, together with their standard deviations are given in table 5.

TABLE 5

Means and standard deviations of the durations of life of the different inbred lines of rats.

LINE	MEAN		STANDARD DEVIATION	
	MALES	FEMALES	MALES	FEMALES
1	233± 9	210± 7	54± 6	56± 5
6	289±18	258±10	90±13	37± 7
7	133± 7	164±11	34± 5	55± 8
8	295±14	221± 9	82±10	46± 7
10	217±11	227±11	54± 8	50± 5
11	132± 6	132± 5	33± 4	35± 4
12	160± 5	158± 6	21± 3	40± 4
13	344±15	274± 9	78±10	49± 6
14	160± 7	151± 5	45± 5	36± 4
15	144± 6	155± 9	43± 4	44± 6
16	143± 7	116± 6	45± 5	40± 4

The data show several significant differences in the average duration of life between the lines. Such differences may be appreciated best by comparing the bar diagrams in figure 3. The height of the bars represents the

average duration of life of the line indicated by number at the base of the bar. The left-hand portion of the bar represents the males; the right-hand portion the females. Evidently lines 6, 8 and 13 survived for the longest period on the vitamin D deficient diet. Lines 1 and 10 lived a shorter time, while the remaining lines showed the poorest survival.

The data show that the two sexes within a given line correspond fairly well in their duration of life. It is likewise evident that the lines which tend to live the longest time are those which show the differences between the sexes.

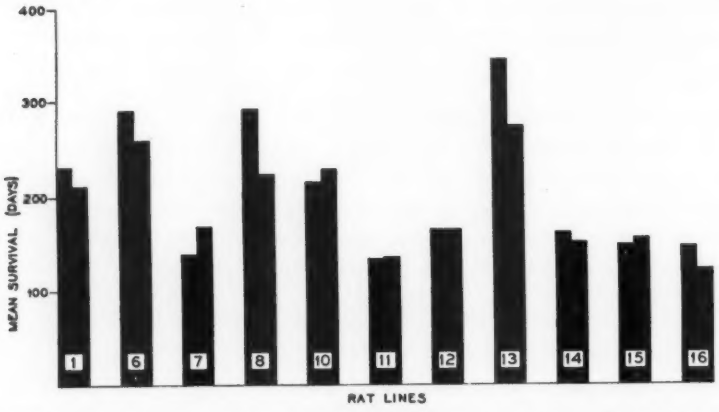

FIGURE 3.—Average duration of life of genetically differentiated lines of rats fed on a diet deficient in vitamin D. Left-hand side of bars, males, right-hand side, females.

The relative effect of inheritance may be more clearly demonstrated by a rather precise comparison. The central idea behind inheritance is that closely related individuals, on the average, will resemble each other more than will unrelated ones. The degree of resemblance between any two relatives may thus be determined as the quantitative difference between the measurements of a particular character in the two individuals. Genetically unrelated animals composing another group may be treated in a similar manner. The difference between measurements for the related and unrelated or random sample groups will give a quantitative value for the effect of the inheritance. If now, we desire a direct measurement of the amount of genetic correlation between the related individuals in the groups the summed squares of these differences will give such a measure of this inheritance effect.

The total squared differences for the durations of life of the whole population of rats on this vitamin D deficient diet may be divided into two parts; the variation due to differences between the different genetic

lines and the variation due to the random variation of the individuals within the lines. Since the genetic differences between the lines are the only known causative agents of variation, this variation may properly be attributed to the influence which the inheritance exerts on the utilization of any available vitamin D and of other elements in the deficient diet. The variation within each line may, for the moment, be considered to be induced by unknown environmental factors. The contribution of these two sets of factors to the total variability is seen below.

Males	
Variation in whole population	1,334,400 days
Between related individuals	848,320 days
Within related lines	486,080 days
Females	
Variation in whole population	690,720 days
Between related individuals	377,120 days
Within related lines	313,600 days

These results show that over half of the sum squares of the durations of life within the whole population are due to the differences between the genetically different lines of rats. For the males the contribution made to the whole variation by these racial differences amounts to 63 percent of the total. For the females the contribution is 55 percent of the total. The statistical significance of these differences may be determined in the usual way by comparing the variance of the between group class with that of the within group class, where the degrees of freedom are 10 for between lines and 159, males, and 156, females, for within the lines. These variances for the males are, between lines 84,832 days; and within lines 3,057 days; and for the females, between lines 37,712 days; and within lines 2,010 days. The variance due to the genetic differences is at least 18 times that within the racial lines where a difference of only 2.5 times would be significant. Both sexes thus agree in showing a well marked difference in the effects of the vitamin D deficient diet in populations of closely related individuals as contrasted with a group bred at random. The differences between the variances of the males and females, while fairly large could easily be due to random sampling since to be significant one difference would need to be more than 5 times the other.

There are three classes of genetic relationship for the individuals within the different racial lines. The first is that between individuals of the same litter; the second that between individuals of the same parents but different litters; the third that between the offspring of one pair with those of another pair of the same line. The variance contributed to the total by these different groupings of the data is found below.

These results are concordant in showing the closely related individuals to be quite similar in their duration of life whether they are born into the same litter, different litters of the same parentage or born of different parents but of the same racial inbred line. The results for the males vary somewhat, those for the females are rather constant, seeming to represent a more nearly average result. The variance within the matings—2,200

	DEGREES OF FREEDOM	SUM OF SQUARES IN DAYS	VARIANCE IN IN DAYS
Males			
Whole population	169	1,334,400	
Between racial lines	10	848,320	84,832
Between different parents but within racial lines	19	82,400	4,320
Between litters of same parents	23	147,120	6,400
Within litters	117	256,560	2,200
Females			
Whole population	166	690,720	
Between racial lines	10	377,120	37,712
Between different parents but within racial lines	19	65,200	3,432
Between litters of same parents	17	47,760	2,808
Within litters	120	200,640	1,672

days for the males and 1,672 days for the females—is a fair estimation of the random variance unaccounted for by the differences in the racial lines. As pointed out earlier, the ratio between this variance and that between the racial groups shows that statistically speaking the odds in favor of an inherited effect on a dietary exhaustion are large indeed. Environmental effects common to the progeny of different parents within racial lines or to litters of the same parents also contribute elements tending to make their duration of life more nearly alike. If the variance of these two groups be compared with that of the within litters group, we find that to be considered significant, the former value should be almost twice the latter. Three out of four groups meet this requirement. The variance of one of the three groups, between litters of the same parents in the males, is nearly three times that of the within litters group. These differences may be considered as indicative of some effect of common environmental or genetic factors (uncontrolled by the previous inbreeding) which were common to these particular groups. The net conclusion to be drawn from the evidence is, however, clear; the life-span of rats confined to the deficient diet is influenced markedly by genetic constitution.

THE INTERRELATION OF CHARACTERS AFFECTING THE LIFE-SPAN

It is of interest to inquire further into the inheritance effect and to determine, if possible, what characters the inheritance may affect and thus

account for the observed differences between the genetic lines. Two such characters which vary with the different inbred lines are known to be present—the size of litter and the ability to reach a given initial weight at 46 days, the age when the deficient diet was commenced. These variables are interrelated. Both could conceivably be responsible for the observed inherited effects if they are found to be correlated with the survival times of the different lines. Furthermore it seems likely that these variables, litter size and weight at 46 days, would themselves be correlated. The correlations are presented below.

Besides the effect on the life-span, four other measures have been used in estimating the effects of the defective diet, maximum weight attained while on the defective diet, weight just prior to death, days between start of diet and maximum weight, and days between maximum weight and death. The interrelations of these variables, as well as their relation to initial weight and litter size, have a direct bearing on the problem in hand.

TABLE 6

Correlations of survival time and other indicated variables for rats on a vitamin D deficient diet

CHARACTERS CORRELATED	CORRELATION COEFFICIENTS	
	MALES	FEMALES
Weight at 46 days and age at maximum weight	0.43±.04	0.29±.05
Weight at 46 days and maximum weight	.82±.02	.83±.02
Weight at 46 days and age at death	.60±.03	.50±.04
Weight at 46 days and last weight	.76±.02	.84±.02
Weight at 46 days and litter size	−.48±.04	−.46±.04
Age at maximum weight and maximum weight	.71±.03	.52±.04
Age at maximum weight and age at death	.85±.01	.76±.02
Age at maximum weight and last weight	.64±.03	.47±.04
Age at maximum weight and litter size	−.23±.05	−.06±.05
Maximum weight and age at death	.78±.02	.68±.03
Maximum weight and last weight	.91±.01	.91±.01
Maximum weight and litter size	−.39±.05	−.28±.05
Age at death and last weight	.67±.03	.58±.03
Age at death and litter size	−.34±.05	−.15±.05
Last weight and litter size	−.33±.05	−.38±.05

Examination of table 6 reveals a close correlation between many of the variables. Such variables as age at maximum weight, maximum weight, last weight, are closely correlated with age at death, the coefficients being quite high, 0.85, 0.78, 0.67, for the males. These variables may be utilized to measure the defects of the diet and they also completely determine such other variables as gain or loss in weight on the deficient diet. This fact makes it entirely proper to confine our consideration of the inheritance to its effects on the life-span, since such other items are in large part only different measures of the same variable. The litter size and individual

weight attained at 46 days, the time when the deficient diet was commenced, are in a somewhat different category in so far as their relation to the effects of the diet on the life-span is concerned. Weight at 46 days is rather highly correlated with the after life-span when the rat is on the deficient diet. The ability to reach a given weight at a given time is also an inherited characteristic. The correlations of table 6 suggest that the inheritance of this character is a contributing cause of the observed inheritance of the length of the life-span between the inbred lines. A similar observation may be made for the litter size. This latter case is somewhat different, however, since litter size may be a variable which expresses its effect entirely through its influence on the weight at 46 days. If this be true, all the attention may be given to the weight at 46 days. The partial correlation coefficients throw some light on the problem. The first order partial correlation coefficient between the weight at 46 days and the life-span where account is taken of the effect of the litter size is $0.53 \pm .04$ for the males, and $0.49 \pm .04$ for the females. The partial correlation coefficient for litter size and the life-span where the effect of the 46-day weight is properly accounted for is $-0.06 \pm .05$ for the males and 0.11 ± 0.5 for the females, correlations which are not statistically significant. The characteristic which accounts for all of the effect of litter size and also contributes something to determining the life-span under the conditions of the experiments is the weight at 46 days. The contribution which the inheritance of this character makes to the length of survival may now be investigated.

ON THE CHARACTER BASIS FOR THE GENETIC DIFFERENTIATION SHOWN IN THE DURATION OF THE LIFE-SPAN

The inbred strains of rats utilized for these experiments differ markedly in their weights at 46 days of age, the period including nursing, weaning and growth. The broad ration which was used has maintained a colony of many rats throughout life and over a period of 15 years. Of the total squared sums of these weights for the whole population of males, 45 percent is contributed by the line differences. For the females the contribution is 57 percent. These contributions are statistically significant since the variance between the lines is for the males 13 times and for the females 19 times those of the variances within the lines.

The question of how much this inheritance is contributory to the inheritance of the duration of life under the unfavorable diet may be analyzed as follows. The sum of the squared differences for the durations of life of all rats was earlier split into two parts; that which is due to the differences between the inbred lines and that found within the lines. The question to be answered is, how much of each of these variations is due to the variations in initial weight? The numerical values necessary to answer

this question may be determined from the correlation coefficients between the weights at 46 days and the life-spans within the two groups, between the inbred lines and within these lines.

These correlation coefficients are equal to 0.84 for the males and 0.62 for the females in the group where the variation is dependent on genetic differences between the inbred lines. Statistically speaking, they are significant. They are not significantly different from each other, however, since the difference is only 0.22 with a probable error of about 0.28. An average value of 0.73 for the correlation coefficient would thus be a fair estimate of the effect of the inheritance of weight on the life-span. The correlation coefficients between weight and duration of life within the inbred lines is the same for both males and females, 0.34. This is also significant since a correlation of only 0.21 would be exceeded by chance but once in 100 trials. This correlation of 0.34 within the inbred lines would seem to be due to two possible kinds of variables; inheritance heterozygosis, which is as yet uncontrolled by the inbreeding, and common environmental factors having common effects on growth and life-span. It is significant to note that correlations due to such causes are only half those due to the controlled genetic causes, showing that to this extent, at least, the inbreeding has segregated important hereditary factors for life-span and weight into fairly pure racial groups.

The amount of the variation in life-span due to differences in the inheritance for weight may be determined from the relation

$$v = V(1 - r^2)$$

when V is the variance of the life span, r is the correlation coefficient between weight at 46 days and the survival time and v the partial variance, that is, the variance remaining after account has been taken of the effect of the inherited variation in weight. By substituting the correlation coefficient of 0.73 in this equation we find that the variance remaining after account is taken of the effect of the weight at 46 days is only 47 percent of that where weight varies as it will. Of that portion of the total variance attributable to inherited differences between the rat strains (59 percent), 53 percent or 31 percent of the total is contributed by inherited differences in weight. The remaining 47 percent of the inheritance effect on duration of life is due to characters which are at present unknown. The same reasoning may be applied to the variation in length of life within the different lines. The variation due to the uncontrolled inheritance of weight within the inbred lines or to common environmental factors is measured by a correlation of 0.34. The variance remaining after proper account is taken of these factors is 88 percent. The portion of the variance within rat strains due to this character, weight, is consequently but 12 percent of that observed within the rat strains. The tabulation of these results is

as follows. The total sum of the squares is reduced to a percentage, 100, and the contribution of each of the different factors to this variation listed as a percentage of this total.

Total variation in life span (sum of squares)	100
Variation between genetically different lines	
Percent of variation due to inheritance of weight	31
Percent of variation due to unknown characters	28
Total attributable to inheritance factors	59
Variation within genetically similar lines	
Percent of variation due to weight	5
Percent of variation due to unknown environmental characters	36
Total attributable to chance inherited and environmental influences	41

TABLE 7

Analysis of the variation of weight at 46 days and life-span in strains in inbred rats.

ORIGIN OF VARIATION	DEGREES OF FREEDOM	WEIGHT AT 46 DAYS (GMS.)		LIFE-SPAN (DAYS)		CORRELATION	
		SUM OF SQUARES	VARIANCE	SUM OF SQUARES	VARIANCE	CROSS PRODUCTS	CORRELATION COEFFICIENT
Male							
Whole population	169	58,010	334.3	1,334,400	7,896	166,630	0.599
Between strains	10	26,073	2,607.3	848,320	84,832	124,210	.835
Within strains	159	31,938	200.9	486,080	3,057	42,420	.341
Females							
Whole population	166	37,785	227.5	690,720	4,161	80,710	0.499
Between strains	10	21,655	2,165.5	377,120	37,712	56,350	.624
Within strains	156	16,130	103.5	313,600	2,010	24,360	.343

In general, the evidence leads to the following conclusions. Since the only known variable which tends to differentiate the inbred lines is heredity, the evidence indicates that somewhat more than half (about 59 percent) of the variation in life-span under conditions made unfavorable by a deficiency of vitamin D in the diet, is due to characters under hereditary control. Approximately 36 percent of the variation is due to unknown environmental influences. Of the half due to heredity, a half of that, or a quarter of the whole, is attributable to the inheritance of the character weight at 46 days. The other quarter, although definitely due to heredity, must be attributable to the inheritance of characters unknown but important to survival under the prescribed dietary conditions. Only 5 percent of the variation within the lines is assignable to weight variations and 36 percent to unknown but nevertheless very real environmental

or uncontrolled hereditary factors. The data from which these comparisons are drawn are given in table 7.

SOME GENERAL BIOLOGICAL ASPECTS OF THE DATA

The data favor the view that within a species the reactions of individuals to apparently essential dietary constituents may differ and that this variation is controlled partly by the inheritance. It is of interest to digress and consider here the consequence of such variation in evolution. In the shifting of species from one locality to another of quite different dietary possibilities, it would seem that extensive modification in the physiological economy of the group would often be required. Such, for instance, would be the case of groups moving from high, dry plateaux over mountains into low, hot, moist regions; or, in the laboratory, in the transfer of bacteria from a susceptible host to culture media and *vice versa*.

Two explanations for such modifications in evolution seem possible. First the group could move into the unfavorable area and through some action of the environment become modified and survive the conditions imposed. These modifications, of course, would have to be hereditary and the objections can be raised that the germ plasm is not readily altered and that only a small proportion of such modifications as do occur improve the survival value of the animal. The chance of establishing the initial foothold in the unfavorable area would thus be slight. An alternative proposition would be that within a group certain animals may become potentially suited in their genetic constitutions to environments different from those in which they are found, but into which they can migrate. These extreme forms which would rise at random and without any evident adaptive relation to the environment would thus be fitted to meet the new environmental conditions and to establish themselves in sufficient numbers to gain a foothold. Further mutation and selection, even though the rate be small and the effect slight, would eventually tend to complete the adaptive change.

The evidence obtained in our experiments points to distinct genetic differences in the dietary requirements of strains of rats. These differences existed before there was any known exposure of the race to dietary deficiencies. Certain lines were adjusted by their previous inheritance to a somewhat lower requirement of given food elements than were others, and could, consequently, meet the lack of such elements more successfully. The fitting of certain members of the group to the environment came first rather than as a consequence of previous modification.

This view is supported in the experiments on disease resistance, in which the different inbred lines of a race are exposed to a disease rare to

it. Examples include pseudorabies of cattle introduced into mice (GOWEN and SCHOTT 1933) or *B. abortus* of swine in rabbits (COLE, 1930), since in both instances it has been possible to demonstrate that certain strains have inherited resistance to the disease, whereas others show distinct susceptibility to it.

Other diseases which are known to be quite dependent upon the genetic constitution of the host for their infectivity do not furnish such critical evidence, since these diseases occur commonly in the species and selection for susceptibility or resistance may have occurred.

Such diseases as rat typhoid, *S. enteritidis* of IRWIN (1929), mouse typhoid *S. aertrycke* of SCHOTT (1932), *B. enteritidis* of WEBSTER (1933), white diarrhea, *S. pullorum* of ROBERTS and CARD (1926), typhoid, *S. gallinarum* of LAMBERT (1932) fall into this group, since it is uncertain whether the fitting of specific groups to the environment came first or as a consequence of previous modification.

The bacteria and especially the protozoa furnish significant evidence of both types of genetic modification of the host organization. Evaluation of the results necessitates a slight reorientation in thought, since the selection may affect the soma instead of the germ plasm in these rapidly reproducing unicellular forms. In certain bacteria, the colon group, for instance, division may occur every 20 minutes. A single organism then, in the course of 8 hours could give rise to 16,000,000 individuals if all survived during the period. Since the possible rate of mutation for a specific gene is 1 to 10 in 10,000,000 individuals nearly every gene and the character or characters which it governs could have changed at least once in 24 generations.

Wild species of paramecium (JENNINGS 1908), difflugia (JENNINGS 1916, 1929), centropyxis (ROOT 1918), yeast (HANSON and others 1906), colon bacillus (BARBER 1907), and many others have shown several biotypes of diverse heredity, each of which is capable of reacting differently to the same or to diverse environments. Experimentally, the environmental agents used were in some cases poisons with which the organism presumably had had no previous contact,—the parasitic trypanosomes (TALIAFERRO 1926) or the free-living paramecia (JOLLOS 1921).

The choice of the original biotype seemed fortunate in certain cases (JENNINGS 1929) since the results were not always reproducible with other stocks. The effects followed a pattern closely similar to that observed in other less prescribed environments, in which for example the criterion was that the animals should reach a given size or survive a certain heat. The initial contrast between the selected lines seemed to be due to previously differentiated biotypes selected from the wild population. Further selection and presumably further purification of these biotypes resulted in further

progress, that is, increase in size or resistance to the heat or poison. If still further progress was attained it occurred in more or less discrete steps of unequal length in both generation time and advance toward the greatest adaptibility for the environment. This goal, as DALLINGER (1887) showed, may be far removed from the ordinary environmental conditions under which the species are accustomed to live: in 7 years time the temperature requirements of flagellates were changed from 60°F. to 158°F., 16°C. to 70°C. But these changes in the genetic structure of the organisms are no more permanent than those found in the wild type from which they started many generations earlier. For, if the environment is now changed, the forms may again, by a slow step-like process, be made to return to forms whose requirements are those of the wild stock from which it originated. This result is not so surprising if cognizance is taken of the relatively large numbers of animals involved, and the rates at which genes vary under natural conditions. It suggests that in any population, as in the rat population here studied, variation in the nutritive requirements, etc., are occurring which are purely outside the requirements of the given environment and that if the environment should change those variants having the capacity to meet the changed conditions become the parents of those which are to form the new race.

SUMMARY

The data presented here show that rats which are fed a low vitamin D, high calcium diet, from the 46th day of age have an average duration of life of 5 to 6 months, or not quite half, that of like animals on a normal diet. Rats on the deficient diet die off more sharply than those fed normally. The standard deviations of duration of life of rats on the deficient diet are less than half those of animals on the normal diet. The relative variation is consequently greater in the normal rats. It is further noted that males on the vitamin D deficient diet live slightly longer than the females, but on a normal diet the results are reversed. The frequency curves of death show a distinct skewness.

Data for the separate lines show distinct differences between these lines in respect to length of life. The variations range from 131 to 345 days for the males and 132 to 267 days for the females. A comparison of the variations found for the whole random bred population and for the rats related by strain, shows that a distinct correlation exists between the duration of life of the animal within a given line. The correlation coefficient for the males is 0.63; that for the females 0.55.

Within the strains there are 3 relationships which bear on the strain difference: correlations between members of the same litter, between individuals of two litters and the same parents, and between individuals of

the same line but of different parents. The analyses of the effects of these relationships are concordant in showing that the genetic differences between the racially differentiated lines account for the greater part of the variation. Variation due to the similarity in environment of offspring of the same parents or of the same litter are rather small and statistically just significant.

The hereditary differences between the inbred strains are found to account for somewhat more than half of the variations observed in the life-span. Of this hereditable variation, about half seems to be due to the inheritance of the character, body weight at 46 days. The remaining quarter of the variation in life-span due to heredity may be attributed to the inheritance of characters as yet unknown but important to survival under the dietary conditions imposed by the experiment. Of the variation remaining after account is taken of the inheritance effect, only 5 percent is attributable to variation in weight. The other 36 percent is due to unknown factors apparently largely of environmental origin, although even here some of this variation could be due to some heterozygosis of the germ plasm as yet uncontrolled by the inbreeding.

Finally, the evidence considered from a broader biological viewpoint has a bearing on the problems of adaptive evolution which may be significant. The suggestion is made that the fitting of certain groups within a species to particular environmental conditions would seem to come first rather than as a consequence of modification by the environment. The experiment presented above supports such a contention, for the evidence points to distinct genetic differences in the dietary requirements of the strains which, so far as it is known, never had been exposed to the particular deficiencies involved.

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THE GENETICS AND PHYSIOLOGY OF SELF-STERILITY IN THE GENUS CAPSELLA

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INTRODUCTION

THE oppositional factor hypothesis of EAST and MANGELSDORF (1925) and FILZER (1926) will account for the genetics of self-sterility in many species of plants, particularly those of the Solanaceae and Scrophulariaceae. When two cross-fertile plants are mated normally, either four intra-sterile, inter-fertile classes will result all of which will be fertile with both parents, or only two such classes will be produced of which one will be fertile with both parents while the other will be fertile with the mother and sterile with the father; in either case, the classes will be of the same size. The class of the mother is never represented in the offspring. Therefore, reciprocal crosses are never alike. Self-sterility in these species is controlled by three or more allelic genes, usually designated s^1, s^2, s^3, etc. Two only are present in the tissues of the style of a normal diploid plant; and when a pollen grain which bears either one of the two is placed upon the stigma, its pollen tube does not grow sufficiently rapidly to reach the ovules before the flower withers. On the other hand, when a pollen grain which carries a self-sterility gene different from either one in the style is placed upon the stigma, growth of the tube proceeds at an accelerated rate and fertilization takes place.

There are other species of plants which are self-sterile but in which the behavior of the intra-sterile, inter-fertile classes is different from that in the species of the Solanaceae and Scrophulariaceae which have been investigated. The author (RILEY 1932) showed that *Capsella* (*Bursa*) *grandiflora* definitely does not behave as it should if the same type of *s* factors controlled self-sterility. In that paper, a possible genetic scheme was suggested, but further work indicates that it must be modified. CORRENS (1912) had described a case of self-sterility in *Cardamine pratensis*, a plant which belongs to the same family as Capsella. He found that two cross-fertile plants produced four intra-sterile, inter-fertile classes; one was sterile with the mother and fertile with the father, another was just the opposite, a third was fertile with both parents and the fourth was sterile with both. The results in Capsella which were reported earlier seemed to indicate an analogy with the situation in Cardamine. In most families,

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only the two classes of the parents were found, but in others, a third class fertile with both parents was present; some families seemed to contain a class sterile with both parents in addition to the two parental classes, while others contained all four classes, as CORRENS had found in Cardamine. An occasional family produced only one class which further complicated the situation. It seemed at the time, that the situation in these two Crucifers was basically the same, but that some modifying condition was present in Capsella; it was the purpose of the further investigation reported in this paper to determine what was the nature of this modifying condition.

Another species which proved to be different from Nicotiana and Veronica and therefore not to be controlled by oppositional factors was *Lythrum salicaria*. DARWIN, BARLOW, EAST and VON UBISH all found that the three types of flowers in this species were relatively intra-sterile; and the latter workers showed that in most plants two gene pairs determine trimorphism. In Lythrum, self-sterility seems to be determined by the same genes *A*, *a*, *B*, and *b* which determine the three forms of the flowers.

MATERIALS AND METHODS

The plants used in this investigation resulted from crosses reported in the previous paper, and the technique of hand pollination was the same. The families of the first generation of these new studies were raised in Doctor G. H. SHULL'S greenhouse at PRINCETON UNIVERSITY, while those of the next two were grown in the greenhouse of the BUSSEY INSTITUTION of HARVARD UNIVERSITY through the courtesy of Doctor E. M. EAST, under whose sponsorship this work was carried out, and under a fellowship from the NATIONAL RESEARCH COUNCIL.

In the earlier paper, the three intra-sterile, inter-fertile classes were designated by the letters A, B, and C, as in tables 3 and 4 of that paper. The method of identifying classes from one generation to another also was given. The classes considered in this paper descended from those of the first paper and the same symbols are used for the three classes. There is an unbroken continuity of classes in the two papers.

OBSERVATIONS

In the previous paper, certain families seemed to be composed of three classes, one of which was sterile with both parents, while some other families consisted of four classes of which one again seemed to be sterile with the classes of both the mother and the father. There was no *a priori* ground for doubting this situation, and as the latter type of family was identical with that found by CORRENS, the tentative explanation offered previously was based upon the assumption that this situation was correct. When families appeared in subsequent generations which seemed to split in the same manner, however, the plants which gave sterile results with

both testers were retested. When the two testers were again crossed on to plants which were sterile with both parents, and if the second tests were made a few weeks after the first trials, it was discovered that these plants set seed with one of the test plants. For example, plant 62(6) had been crossed with plant 62(1) and set no seeds in nine capsules; with 62(5) no seeds were produced in six capsules. As plants 62(1) and 62(5) were fertile together and therefore of different classes, plant 62(6) seemed to be sterile with both testers. Plants 62(1) and 62(5), by appropriate tests, were shown to be of the same classes as their parents, so that family 62 seemed to segregate into three classes of which two resembled the parents while the third was sterile with both parents. The cross, 62(6)×62(1), had been made in the beginning of December 1931 when the plants had just begun to bloom, and the other cross, 62(6)×62(5), had been made a few weeks later. On March 1, these crosses were repeated when the plants were mature but still in good condition. The cross 62(6)×62(5) again yielded no seeds in seven pollinations, while the other cross, which had previously been classed as sterile, produced 45 seeds in five pollinations, or an average of nine seeds per capsule. After this was established, all plants which seemed to be sterile with both parents and which could be retested were retested later, and in all cases one of the crosses which had been sterile proved to be fertile upon repetition. It was impossible, of course, to repeat crosses upon plants which had given similar results in previous generations, but it is probable that if such could be done, these plants also would prove to be fertile with one of the testers and therefore with one of the parents. This discovery threw a new light upon the situation. Just what determines this early sterility in such cases has not been determined, but it is probably not due to self-sterility. It is reasonable, then, to reexamine the data, discarding as insufficiently tested all plants in the earlier generations which seemed to be sterile with both parents. When this is done, it is seen that these families split into two groups which are like the parents or into three of which two are like the parents and the third fertile with both parents, or that only one class is present and that it is sterile with one parent and fertile with the other. For example, family 2 of the previous paper seemed to be identical with CORRENS's family, segregating into four classes; but if the one plant which was sterile with both testers is disregarded, the family splits into three classes of five, two, and four plants, respectively.

When all the families reported previously are reconsidered, assuming that plants recorded as sterile with both parents appear to be so because of a technical error, it is seen that most families segregate into two groups, but that four families consist of all three groups and that these families came from the cross A×C. It was the first purpose of this investigation to trace such families further.

Family 39 was one family that split into three classes, and intra-family crosses were tested the following year. Family 62 from the cross 39(5)×39(1), or Class B×Class A, contained five plants of Class A and five of Class B. Family 63 arose from 39(9)×39(1). Plant 39(9) was Class C, and this cross resulted in four plants of Class A, four of Class C and one of Class B. Family 64, from 39(9)×39(5), contained five plants of Class C and five of Class B. The parents were Classes C and B. Family 39, then, consisted of classes A, B, and C. Class A×Class B gave the same two classes as the parents, and the same was found to be true when the parents were of Classes B and C. However, when Classes A and C were intercrossed, a family was produced which consisted of all three classes.

Although this result began to make the situation much clearer, it was considered advisable to study the classes which are produced when self-sterile segregates from crosses between self-sterile and self-fertile plants are crossed with other plants of the self-sterile species. This point was tested in 1932. Families 69, 70, 71 and some plants of 72 served as test material to identify the classes of these hybrids.

Family r3 was the F_1 between a Class C plant of *C. grandiflora*, 26502(7), and a plant of the self-fertile species *C. rubella*. Self-fertility is dominant to self-sterility (RILEY 1934), and four plants of family r3 were selfed to produce an F_2. All four resulting families split. The self-steriles of three of these families were all of Class B, while those of the fourth, r6, were Class C. A heterozygote of family r6 was selfed to produce family r24. Plant r24(15), a self-sterile of Class C, was crossed with 63(6), which was of Class A, reciprocally. The resulting families, r35 and r37, split into Classes A and C. When r24(15) was crossed with a Class B plant, twelve plants of Class C resulted and none of Class B.

A cross between a Class B plant, 26502(6), and the self-fertile *C. tuscaloosae* resulted in an F_3 family, t26, in which all the self-sterile segregates were of Class B. Plant t26(3) was crossed reciprocally with plant 60(1) (Class C) and Classes B and C were present in each resulting family.

To omit a lengthy discussion of the families, most of the families of *C. grandiflora* and of hybrids between this species and the three self-fertile species which are discussed in this and the previous paper are listed in table 1. This table includes the origin of the families, the genotypes of the parents according to the theory outlined in the next section of this paper, and the classes expected and those obtained in the offspring.

DISCUSSION

Before this more recent work, it appeared that self-sterility in Capsella was determined by the same type of gene that appears to control the situation in Cardamine, but that balanced lethals with crossing-over

TABLE 1

Listing the families of Capsella grandiflora and some of the famili of the self-fertile species and hybrids of the entire study, with their origin in terms of self-sterility genes and the expected and obtained classes.

FAMILY	PARENTS	GENOTYPES OF PARENTS	CLASSES EXPECTED	CLASS OBTAINED
1	26674(18)×26674(13)			7A:4B
2	26669(50)×26669(49)			5A:4C:2B
3	26664(35)×26664(31)			2A:1C
4	26502(7)×26502(6)	$uS^cs \times uss$	1C:1B	1C:4B
5	26502(6)×26502(7)	$uss \times uS^cs$	1C:1B	4C:2B
7	2(2)×1(1)	$uS^cS^c \times Tss$	1A:1C	5A:3C
8	2(10)×1(11)	$uS^cs \times uss$	1C:1B	7C:3B
9	5(3)×2(12)	$uss \times Tss$	1A:1B	7A:4B
10	2(2)×1(2)	$uS^cS^c \times uss$	All C	10C
11	2(12)×1(11)	$Tss \times uss$	1A:1B	6A:4B
12	2(10)×1(1)	$uS^cs \times Tss$	2A:1C:1B	Insuff. tested
13	5(3)×2(10)	$uss \times uS^cs$	1C:1B	2C:7B
14	2(4)×1(2)	$uS^cs \times uss$	1C:1B	8C:6B
15	5(6)×4(5)	$uS^cs \times uss$	1C:1B	8C:2B
17	5(5)×2(12)	$uS^cs \times Tss$	2A:1C:1B	3 classes
18	5(6)×2(12)	$uS^cs \times Tss$	2A:1C:1B	3 classes
29	3(3)×1(1)	$uS^cS^c \times Tss$	1A:1C	2 classes
30	3(3)×2(7)	$uS^cS^c \times uss$	All C	10 C
31	4(3)×1(11)	$uS^cs \times uss$	1C:1B	3C:7B
33	5(1)×4(5)	$uS^cs \times uss$	1C:1B	6C:4B
34	8(1)×8(5)	$uS^cs \times uss$	1C:1B	4C:5B
35	8(5)×8(1)	$uss \times uS^cs$	1C:1B	4C:6B
36	9(2)×12(1)	$uss \times Tss$	1A:1B	6A:4B
37	12(1)×9(2)	$Tss \times uss$	1A:1B	5A:5B
38	9(6)×10(1)	$Tss \times uS^cs$	2A:1C:1B	5A:3C:1B
39	10(1)×9(1)	$uS^cs \times Tss$	2A:1C:1B	8A:1C:1B
42	8(5)×9(1)	$uss \times Tss$	1A:1B	6A:4B
43	8(5)×14(9)	$uss \times uS^cs$	1C:1B	3C:7B
44	9(2)×9(1)	$uss \times Tss$	1A:1B	8A:2B
45	9(3)×9(1)	$uss \times Tss$	1A:1B	5A:5B
49	5(3)×2(12)	$uss \times Tss$	1A:1B	6A:4B
51	7(4)×7(1)	$uS^cs \times TtS^cs$	4A:3C:1B	3A:5C
52	7(9)×7(1)	$uS^cs \times TtS^cs$	4A:3C:1B	3A:7C
53	2(2)×1(1)	$uS^cS^c \times Tss$	1A:1C	7A:2C
56	2(10)×1(11)	$uS^cs \times uss$	1C:1B	7C:3B
59	10(1)×9(2)	$uS^cs \times uss$	1C:1B	3C:7B
60	35(2)×35(5)	$uss \times uS^cs$	1C:1B	4C:3B
61	36(3)×36(4)	$Tss \times uss$	1A:1B	5A:4B
62	39(5)×39(1)	$uss \times Tss$	1A:1B	5A:5B
63	39(9)×39(1)	$uS^cs \times Tss$	2A:1C:1B	4A:4C:1B
64	39(9)×39(5)	$uS^cs \times uss$	1C:1B	5C:5B
65	42(2)×42(1)	$Tss \times uss$	1A:1B	7A:3B
68	59(2)×59(1)	$uS^cs \times uss$	1C:1B	3C:3B
69	60(2)×60(1)	$uss \times uS^cs$	1C:1B	2C:5B
70	61(3)×61(1)	$Tss \times uss$	1A:1B	5A:5B
71	62(1)×62(5)	$Tss \times uss$	1A:1B	7A:3B
72	63(4)×63(1)	$uS^cs \times Tss$	2A:1C:1B	insuff. tested

TABLE 1. (*Continued*)

FAMILY	PARENTS	GENOTYPES OF PARENTS	CLASSES EXPECTED	CLASSES OBTAINED
3333	60(7)×60(1)	*tss*×*tS^{c}s*	1C:1B	4C:6B
3334	61(8)×61(3)	*tss*×*Tss*	1A:1B	5A:4B
3335	69(2)×69(1)	*tS^{c}s*×*tss*	1C:1B	8C:2B
3336	70(9)×70(1)	*Tss*×*tss*	1A:1B	10A:1B
3337	2(11)×1(11)	*TtS^{c}S^{c}*×*tss*	1A:1C	4A:2C
3338	2(12)×1(11)	*Tss*×*tss*	1A:1B	5A:3B
3342	39(3)×39(5)	*TtS^{c}s*×*tss*	2A:1C:1B	1A:9C:10B
3343	39(4)×39(5)	*Tss*×*tss*	1A:1B	6A:10B
3344	39(6)×39(5)	*Tss*×*tss*	1A:1B	8A:7B
3345	39(7)×39(5)	*Tss*×*tss*	1A:1B	3A:10B
3348	63(9)×63(2)	*Tss*×*tS^{c}s*	2A:1C:1B	5A:12C
3349	70(3)×69(2)	*Tss*×*tS^{c}s*	2A:1C:1B	12A:3C:5B
r3	26502(7)×26322	*tS^{c}s*×*tS^{j}S^{j}*	self-fertile	self-fertile
r7	r3(2)×self	*tS^{j}s*×self	self-steriles=B	1B identified
r6	r3(4)×self	*tS^{j}S^{c}*×self	self-steriles=C	9C
r8	r3(5)×self	*tS^{j}s*×self	self-steriles=B	1B identified
r12	r3(3)×self	*tS^{j}s*×self	self-steriles=B	1B identified
r24	r6(56)×self	*tS^{j}S^{c}*×self	self-steriles=C	1C identified
r30	r24(3)×self	*tS^{j}S^{c}*×self	self-steriles=C	6C identified
r32	r24(5)×self	*tS^{j}S^{c}*×self	self-steriles=C	20C identified
r34	r24(9)×self	*tS^{j}S^{c}*×self	self-steriles=C	3C identified
r35	63(6)×r24(15)	*Tss*×*tS^{c}S^{c}*	1A:1C	4A:2C
r36	r24(15)×64(1)	*tS^{c}S^{c}*×*tss*	All C	12C
r37	r24(15)×63(6)	*tS^{c}S^{c}*×*Tss*	1A:1C	3A:2C
3350	r32(1)×70(7)	*tS^{c}S^{c}*×*tss*	All C	24C
3351	r32(1)×r35(1)	*tS^{c}S^{c}*×*TtS^{c}s*	1A:1C	14A:6C
t1	26502(6)×26323	*tss*×*tS^{j}S^{j}*	self-fertile	self-fertile
t12	t1(3)×self	*tS^{j}s*×self	self-steriles=B	1B identified
t26	t12(16)×self	*tS^{j}s*×self	self-steriles=B	8B identified
t36	t26(11)×self	*tS^{j}s*×self	self-steriles=B	1B identified
t37	t26(13)×self	*tS^{j}s*×self	self-steriles=B	10B identified
t38	t26(15)×self	*tS^{j}s*×self	self-steriles=B	1B identified
t47	60(1)×t26(3)	*tS^{c}s*×*tss*	1C:1B	6C:4B
t48	61(3)×t26(3)	*Tss*×*tss*	1A:1B	4A:4B
t49	t26(3)×60(1)	*tss*×*tS^{c}s*	1C:1B	4C:2B
t50	t26(3)×61(3)	*tss*×*Tss*	1A:1B	4A:2B
3352	t48(1)×t49(4)	*Tss*×*tS^{c}s*	2A:1C:1B	10A:3C:5B
v1	26502(7)×26324	*tS^{c}s*×*tS^{j}S^{j}*	self-fertile	self-fertile
v2	v1(2)×self	*tS^{j}s*×self	self-steriles=B	9B identified
v3	v1(6)×self	*tS^{j}S^{c}*×self	self-steriles=C	7C identified

might account for the small numbers of certain classes in some families. To check this point, counts were made of the number of seeds and of aborted ovules per capsule and of seed germination to learn whether 50 percent seed abortion due to balanced lethals took place. The percentage of viable seed to aborted ovules was extremely variable (from about 16 percent to 90 percent) and there were no indications that balanced lethals were in operation.

The realization that certain classes seemed to be sterile with both parents because of an error in technique, particularly due to pollination when the female plant was too young, made untenable any theory analogous to that of CORRENS; another explanation was sought. To account for self-sterility in Capsella, it must be borne in mind that the self-sterile species is a diploid (n=8) and not a tetraploid, as are *C. bursa-pastoris*, the common species of Shepherd's-purse, and most other species of the genus. *C. rubella*, *C. tuscaloosae*, and *C. Viguieri* are also diploids. Chromosome counts by HILL (1927) and MANTON (1932) established this, but confirmation for this material was sought by examining a number of plants during the last few generations. All showed that the 2n number is 16. The chromosomes are small, and good meiotic figures are few and difficult to obtain, but the diploid nature of these plants is unmistakable.

In formulating an explanation for the genetic situation in Capsella, several important results must be considered. In the first place, when one of the parents was of Class A, this class always appeared in the progeny. This was not true of Class B, for B×C gave only C in several cases of rather large families. Further, when Class B was crossed to a self-fertile, the F_2 self-sterile segregates were all of Class B, while Class C, when mated with a self-fertile, produced F_2 families in which all the self-steriles were either Class B or Class C. These observations can be accounted for by supposing that the sporophytic nature of any two plants determines whether they will be mutually sterile or fertile, and that self-sterility and cross-sterility are controlled by two pairs of genes. The best assumption is that all plants of Class A are sterile together because they possess a dominant gene, *T*. Any two plants having this gene will be reciprocally cross-sterile, but any plant which lacks *T* and is therefore *tt* will be fertile with any plant which has *T*. From this it is seen that *T* must always exist in a heterozygous state, since a plant bearing it can set seed with *tt* plants only. Classes B and C must be *tt* in order to be fertile with Class A, and the differential in this case is another pair of genes which will be designated S^e and *s*. The gene *T* is epistatic to these others. In the absence of *T*, plants which bear the dominant gene of this second pair will be reciprocally cross-sterile and will belong to Class C. Finally, the ultimate recessive, *ttss*, will represent the genotype of Class B plants. In the absence of either dominant gene the combination *ttss* acts to produce incompatibility with all other plants of the same genetic constitution.

If this assumption be correct, Class A×Class B and the reciprocal will reproduce the same two classes if the genotypes of the parents are *Ttss* and *ttss*. However, the plants of Class A may bear the gene S^e in homozygous or heterozygous condition since it is hypostatic to *T*, and if a plant of that nature is mated with one of Class B, Class C will appear in the

progeny. In some cases, Class B × Class C produced both parental classes, while in several families, only Class C was found. This would be expected if the Class C plants had as their respective genotypes *tiS^{c}s* and *tiS^{c}S^{c}*. If a Class A plant which is *TiS^{c}s* is mated with a plant of Class C (either *tiS^{c}S^{c}* or *tiS^{c}s*), the cross will be fertile in spite of the presence of the *S^{c}* gene in both plants, since *T* is epistatic to *S^{c}* and completely nullifies the action which the *S^{c}* gene would have in the absence of *T*. Since *T* cannot be obtained in a homozygous state, there are six possible genotypes which represent the three intra-sterile, inter-fertile classes in Capsella. The results obtained from all the possible combinations of these genotypes are shown in table 2.

TABLE 2

Intra-sterile, inter-fertile classes of C. grandiflora with their genotypes and the results of all the possible combinations between these genotypes.

	CLASS A			CLASS C		CLASS B
	TiS^{c}S^{c}	*TiS^{c}s*	*Tiss*	*tiS^{c}S^{c}*	*tiS^{c}s*	*tiss*
TiS^{c}S^{c}	S	S	S	F	F	F
TiS^{c}s	S	S	S	F	F	F
Tiss	S	S	S	F	F	F
tiS^{c}S^{c}	F	F	F	S	S	F
tiS^{c}s	F	F	F	S	S	F
tiss	F	F	F	F	F	S

There are eleven possible combinations of these genotypes which will be fertile, excluding reciprocal crosses, since they always give the same results. Of these possibilities, six had been tested before 1933. The possible combinations and the families which illustrate them are tabulated in table 3. The only families which were tested sufficiently and which did not split into the classes expected according to the nature of their parentage were families 51 and 52. These arose from crosses within family 7 which in turn came from the cross 2(2) × 1(1), or Class C × Class A. Plant 2(2), when mated with Class B [plant 1(11)] produced family 10, which was composed of Class C plants only, so that plant 2(2) must have had the constitution *tiS^{c}S^{c}*. When mated with 1(1), it should have produced Classes A and C in family 7, which it did; these plants should have been *TiS^{c}s* and *tiS^{c}s*. When two cross-fertile plants of family 7 were crossed, the offspring should have been Classes A, B and C. In both 51 and 52, however, only A and C were found. The only explanation that can be offered is that when the two test plants of the families were crossed onto their sibs, the one or two plants which were of Class B were too young and appeared to give sterile results with the A or C testers instead of being

fertile with both. The cross 51(4)×51(1) produced one seed in seven capsules and possibly would have been fertile had the cross been made later in the life of the plant. Had this occurred, plant 51(4) would have been recognized as belonging to Class B. The same is true of plants 52(5) and 52(8). The complicating sterility condition mentioned earlier in this paper is probably the reason why only two classes were identified in these two families.

TABLE 3

Possible combinations of genotypes giving fertile results, the genotypes and phenotypes of the offspring and all the families which illustrate them.

PHENOTYPES OF CROSS	GENOTYPES OF CROSS	GENOTYPES OF OFFSPRING	PHENOTYPES OF OFFSPRING	FAMILIES
A×B	$TtS^cS^c \times tts$	$TtS^cs + ttS^cs$	A+C	3337;
A×B	$TtS^cs \times tts$	$TtS^cs + Ttss + ttS^cs + ttss$	A+B+C	3342;
A×B	$Ttss \times tts$	$Ttss + ttss$	A+B	1; 9; 11; 36; 37; 42; 44; 45; 49; 61; 62; 65; 70; 71; t48; t50; 3334; 3336; 3338; 3343; 3344; 3345;
A×C	$TtS^cS^c \times ttS^cS^c$	$TtS^cS^c + ttS^cS^c$	A+C	none;
A×C	$TtS^cS^c \times ttS^cs$	$TtS^cS^c + TtS^cs + ttS^cS^c + ttS^cs$	A+C	none;
A×C	$TtS^cs \times ttS^cS^c$	$TtS^cS^c + TtS^cs + ttS^cS + ttS^cs$	A+C	3351;
A×C	$TtS^cs \times ttS^cs$	$TtS^cS^c + TtS^cs + Ttss + ttS^cS^c + ttS^cs + ttss$	A+B+C	2;
A×C	$Ttss \times ttS^cS^c$	$TtS^cs + ttS^cs$	A+C	7; 53; r35; r37;
A×C	$Ttss \times ttS^cs$	$TtS^cs + Ttss + ttS^cs + ttss$	A+B+C	18; 38; 39; 63; 3349; 3352;
B×C	$tts \times ttS^cS^c$	ttS^cs	C	10; 30; r36; 3350;
B×C	$tts \times ttS^cs$	$ttS^cs + ttss$	B+C	4; 5; 8; 13; 14; 15; 31; 33; 34; 35; 43; 56; 59; 60; 64; 68; 69; t47; t49; 3335

In order to test the validity of the theory outlined above, an additional generation was raised in 1933–34, with the families so set up as to produce definitely predictable classes. Families 3333, 3334, 3335 and 3336 were used as test families, producing Classes A, B, and C which were used to identify the classes of the other families. Family 3337 came from the cross 2(11)×1(11), or Class A×Class B. As these seeds were old, the percent of germination was poor and only six plants reached maturity. Four were Class A and two were Class C, which would indicate, in spite of the small population, that plant 2(11) was TtS^cS^c. This is quite probable if one examines the origin of family 2. Tests of plant 2(12) had indicated that it was *Ttss* (Class A). A cross between this and the Class B plant, 1(11),

produced family 3338. From its origin, it was expected to split into Classes A and B only. There were five plants of Class A and three of Class B. A similar set-up produced families 3343, 3344 and 3345. In all these families, the Class B parent was plant 39(5), which, it will be remembered, belonged to a family which had segregated into all three classes. When crossed with three plants of Class A, plant 39(5) produced only Classes A and B in families 3343, 3344 and 3345.

Family 3342 is interesting. The parents were 39(3) and 39(5), or Class A and Class B. Twenty plants were raised, and tests showed that one was of Class A, ten of Class B and nine of Class C. When one parent of a cross is Class A, this class invariably appears in the progeny because of the epistasis of the gene *T* which is present in plants of Class A. The small size of this class in family 3342 was probably the result of test pollinations upon Class A plants in this family when the plants were too young to give their mature sterility reactions. The splitting of this family into all three classes from the cross of A × B indicates that the plant of Class A which was used as a parent had the genetic constitution *TtS^{c}s*, which is perfectly in accord with expectations considering the origin of family 39.

The only family of this series which did not give the expected classes was family 3348. The parents of this family were 63(9) (Class A) and 63(2) (Class C). From the origin of family 63, the plants of Class A had the genetic constitution of *TtS^{c}s* or *Ttss*, while all Class C plants were *ttS^{c}s*. Irrespective of which type of plant of Class A was selected, the progeny should have produced all three classes, A, B, and C. In family 3348, however, there were five plants of Class A and twelve of Class C, but none of the other expected class, B. Undoubtedly, this absence of plants of Class B is due to the action of this peculiar complicating sterility which has been described. The presence of Class C in such unexpectedly large numbers indicates that some plants classed as belonging to this class were probably Class B plants which showed sterility with the Class C tester because of this juvenile sterility. There was no time to repeat the crosses. It is this complicating condition which has made an analysis of genetics of self-sterility in this species so difficult.

Plant 70(3) was a plant of Class A which must have been homozygous for the gene *s*, according to the origin of the family. This was crossed with plant 69(2), a plant of Class C with the constitution *ttS^{c}s*. Such a cross should have produced Classes A (*TtS^{c}s* and *Ttss*), B (*ttss*), and C (*ttS^{c}s*). This cross produced family 3349, and tests upon this family showed that there were twelve plants of Class A, five of Class B, and three of Class C. This was the expected result, and the numbers of each class are not far from the theoretical, which would be 10A:5B:5C.

Two families used for these corroboratory tests were from crosses in-

volving the F_4 self-sterile segregate r32(1). Family r32 originated from a selfing of the heterozygous self-fertile plant r24(5) which had come down from a cross between *Capsella grandiflora* (Class C) and a plant of *C. rubella*. Twenty self-sterile segregates of family r32 were all of Class C and the heterozygous parent was therefore ttS^1S^c. Self-fertility is dominant to self-sterility. The evidence for the location of the factor for self-fertility is not so conclusive as it would be had self-fertile plants been crossed with self-steriles of Class A, but from the data on hand, it can be assumed that this gene is an allele of the S^c-s series and dominant to both S^c and s. Also, it seems to be epistatic to the gene T. Plant r32(1) was undoubtedly ttS^cS^c as it is a self-sterile segregate from the self-fertile plant whose genetic constitution was ttS^1S^c. Plant r35(1) (Class A) has been shown to have come from a self-sterile segregate of Class C crossed with a pure *C. grandiflora* plant of Class A, and plant r35(1) was TtS^cs in constitution. A cross between it and r32(1) produced family 3351 which split into fourteen plants of Class A and six of Class C as expected. When the same female plant, r32(1) was crossed by a plant of Class B,70(7), only plants of Class C should be expected, and they should have the genes ttS^cs. Family 3350 arose from such a cross and was composed of 24 plants all of which were of Class C.

The final family which was examined in this generation was 3352, and it resulted from seeds of the cross t48(1)×t49(4). Family t48 resulted from a cross between a plant of Class A which was homozygous for s and a self-sterile segregate which was $ttss$. Therefore, plant t48(1), since it was of Class A, had the constitution $Ttss$. Family t49 came from the same plant of Class B which produced t48 and a plant of Class C, which had the constitution ttS^cs. Plant t49(4) was ttS^cs. When the cross t48(1)× t49(4) was made, it was predicted that the offspring would split into three classes, since $Ttss \times TtS^cs$ should give Class A (TtS^cs and $Ttss$), Class B ($ttss$), and Class C (ttS^cs). In family 3352, there were ten plants of Class A, five of Class B and three of Class C, which is not far from the expected numbers 9, 5 and 4.

These more recent tests show that the explanation of a two-factor situation with one gene epistatic to the other pair and of the determination of self-sterility, cross-sterility and cross-fertility by the sporophytic nature of both parents is a tenable theory. As regards the genetics of self-sterility in *C. grandiflora*, the same type of genes appears to be present as have been found to control both heterostyly and self-sterility in *Lythrum salicaria* (East 1927). In the latter case, the genes *A*, *a*, *B* and *b* produce two results, one morphological (heterostyly) and the other physiological (self-sterility). The same kind of genes in Capsella, however, has no morphological effect and produces only self-sterility.

In this study on Capsella, the size of the individual families is small, but in several cases, the same type of cross produced a number of families. In table 3, the families produced by the various combinations of genotypes are listed. The summation of the numbers in each class in all the families of several matings are cited below.

Ttss×*ttss*	(22 families) = 130A:97B.	Expected	113.5:113.5
Ttss×*ttS^{c}S^c*	(4 families) = 19A: 9C.	Expected	14 : 14
Ttss×*ttS^{c}s*	(5 families) = 39A:14C:13B.	Expected	33 : 16.5:16.5
ttss×*ttS^{c}s*	(20 families) = 92C:87B.	Expected	89.5: 89.5

While the deviations from the expected are not large, it is suggestive that the double recessive class, *ttss*, is below expectation in all three types of mating where it has appeared. Class A, which contains the epistatic factor, *T*, exceeds expectation in the three types of mating in which it is involved.

PHYSIOLOGY OF SELF-STERILITY

In the previous paper to which frequent reference has been made (RILEY 1932), it was suggested that self-sterility was due to a failure of the pollen to germinate in an incompatible mating. Further studies corroborate this finding. In fertile crosses, the pollen tubes can be seen leaving the pollen grains and passing among the large cells at the top of the stigma; it is difficult to trace the tubes further, but there is no doubt that the pollen grains have germinated. In a sterile combination, the picture is very different. About ninety-nine percent of the pollen grains fail completely to germinate; those that do germinate, send out short tubes which never elongate and which flatten out against the cells of the stigma without growing down among them, or which in some instances grow away from the stigma as is illustrated in figure 1.

In Nicotiana, the pollen germinates in all self- and cross-pollinations. In fertile combinations, the rate of pollen-tube growth is accelerated by chemical substances secreted by the pistil (EAST and PARK 1918), while in sterile matings, the growth curve is depressed after twenty-four hours (EAST 1934). In *Petunia violacea*, YASUDA (1934) showed that self-sterility is caused in part by the failure of pollen to germinate, but chiefly by differential pollen-tube growth.

EAST (1934) maintains that substances are present in the stigma of the mature flower which react with substances in the pollen tubes, resulting in a slowing up of the growth of the tubes in a sterile combination. In most plants, these substances are not present in the young bud, and appear during the twenty-four hours just preceding the opening of the flower. In certain self-sterility genotypes, these substances are developed even earlier, and no bud pollinations can be procured. The behavior of pollen

grains in self-sterile matings of Capsella is different from the situation in Nicotiana, but this difference is probably not a fundamental one. In Nicotiana (EAST 1934), these substances appear to be present in a certain region of the stigma. When the tubes reach this place, their growth-rate slows up markedly, but after this zone of interference is passed, the growth curve again approaches a straight line. In Capsella, it seems that the same sort of reaction occurs, but that the zone of interference is located at the

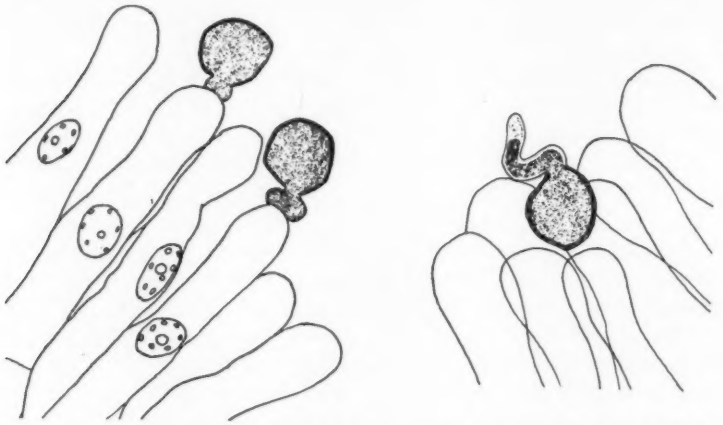

FIGURE 1.—Stigmatic hairs of *Capsella grandiflora*, showing abnormal pollen germination in incompatible matings: (Left) pollen tubes flattened against the stigmatic hairs; (Right) a pollen tube growing away from the stigma. Such cases are exceptional, for most pollen grains do not germinate in an incompatible combination. Aceto-carmine smears, drawn with camera lucida, at table level with 40 objective and 15× ocular.

very end of the stigma in the stigmatic hairs. Instead of slowing up pollen tubes which had begun active growth, the stigmatic substances are produced at such a place in the pistil that the grains do not germinate or at most produce tiny, abortive tubes. It is probable that not only are these substances located higher in the stigma, as the non-germination of most of the pollen grains would indicate, but that they are more powerful, for even when a few tubes do germinate, they cannot enter the stigma.

In Nicotiana, self-sterility is determined by the diploid or sporophytic nature of the female and the haploid or gametophytic nature of the male. If a plant has the genes s^1 and s^2, secretions reflecting their nature would be present in the tissues of the style. If the pollen grains from a plant whose genetic constitution is s^1s^3 are placed upon this stigma, the s^3 grains send out tubes which are accelerated in growth and which eventually fertilize the ovules. The grains which bear the s^1 gene also germinate, but there is

a reaction between the pollen-tubes which come from them and the substance in the style which is produced by the s^1 gene in the female plant. This reaction has a depressive effect on the growth-rate of the tubes. The tubes which bear the s^1 gene are solely s^1 in nature. The pollen mother cells of this s^1s^3 plant were s^1s^3 and the pollen grains have either the s^1 or s^3 genes. Their pollen-tubes are either s^1 or s^3 in reaction. It is reasonable to assume that when the nuclear membrane disappeared during the first division of the microspore bearing the s^1 gene, substances secreted by this gene diffused out into the cytoplasm so that the cytoplasm shows the reactions characteristic of this gene.

In *Capsella grandiflora*, self-sterility, cross-sterility, and cross-fertility are determined by the sporophytic nature of both the male and female plants, as is also true of Lythrum. If a plant has the genes ttS^cS^c, it behaves like a plant of Class C and is sterile with all other plants bearing the genes tt and S^c in combination. When a Class C plant of the genetic constitution ttS^cs undergoes reduction, the microspores are either tS^c or ts. If the pollen grains of this plant are placed upon the stigma of the ttS^cS^c plant, those whose constitution is tS^c fail to germinate, but those which are ts also do not germinate in spite of the absence of the S^c gene in their make-up. On the other hand, pollen grains bearing the genes ts and which came from the B Class ($ttss$) will germinate readily. A plant with the constitution TtS^cS^c will behave as a plant of Class A. If this is crossed by another plant of Class A, the genotype of which is $Ttss$, the result will be sterility although the individual pollen grains of this second plant will be either Ts or ts. The ts pollen, on the other hand, from a $ttss$ or a ttS^cs plant will germinate on and produce seeds with the plant TtS^cS^c. The pollen mother cells of the $Ttss$ plant will undergo reduction to form grains of which the genes will be Ts or ts, but the cytoplasm of these grains will react alike. It is plausible to assume, then, that at the first meiotic division, when the nuclear membrane is broken down, substances produced by the T gene disseminate into the cytoplasm so that at telophase the nuclei are Ts or ts as to their genes, while the cytoplasm of all the cells is T in its reacting properties. This cytoplasmic nature persists throughout the ensuing divisions after the first meiotic division, so that all the male gametophytes have cytoplasm which reacts like that of the sporophyte from which the gametophyte developed. For that reason, the pollen grains which have only ts genes but which came from a sporophyte of the constitution $Ttss$ react like the Ts pollen grains from the same sporophyte, but react entirely differently from ts pollen grains from a $ttss$ sporophyte. The nature of the reacting cytoplasm of pollen grains in Capsella, therefore, is determined probably by a release of nuclear material during the last cell division of the sporophyte generation and the persistence of its ability to function.

SUMMARY

1. Three intra-sterile, inter-fertile classes only have been found in *Capsella grandiflora* and are designated as Classes A, C and B.

2. Class A×Class C will produce Classes A and C or Classes A, B and C, but never Classes A and B only. Class A×Class B will produce Classes A and B or Classes A, B and C or Classes A and C. Class B×Class C will produce Classes B and C or Class C only, but never a plant of Class A. Reciprocal crosses are always alike and produce the same classes in every case.

3. When a plant of *C. grandiflora* of Class B is crossed with a self-fertile species, all the self-sterile segregates in the F_2 are of Class B. When Class C is mated with a self-fertile plant, the self-sterile F_2 plants in some families will be Class C and in others will be Class B, but all the self-steriles in any one family are of the same class.

4. Self-sterility appears to be due to two pairs of genes, *Tt* and $S^c s$. *T* is epistatic to S^c. All Class A plants bear the gene *T* and are sterile together reciprocally, irrespective of the nature of the other genes. Since fertile results will be produced only when a *T* plant is crossed with *tt*, no plants homozygous for *T* are found. Class C plants are all *tt* and bear the gene S^c which may be homozygous or heterozygous. All plants with either one or two doses of this gene will be mutually inter-sterile. Plants of Class B are *ttss* and are all sterile together. The sporophytic nature of the two plants entering into a cross determines whether or not seeds will be produced. From available data, self-fertility appears to be caused by a gene S^f which forms a third member of the allelic series S^f-S^c-*s* and which is dominant to either of the other two genes. It also appears to be epistatic to the gene *T*.

5. Pollen grains bearing the genes *ts* will be sterile upon a plant of the nature TtS^cS^c if they came from a plant whose genetic constitution was *Ttss* or TtS^cs, but will be fertile upon TtS^cS^c if the plant from which they were derived was *ttss* or ttS^cs. This and other evidence indicate that there is a release of nuclear material into the cytoplasm during the reduction division of the pollen mother cells and which shows its effect in the cytoplasm of the mature pollen grain.

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CYTOGENETICS OF HYBRIDS BETWEEN ZEA MAYS AND EUCHLAENA MEXICANA[1]

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INTRODUCTION

UNDOUBTEDLY specific and generic as well as lesser differences may be largely attributed to differences in the chromosomes. Detailed analysis of gene and chromosome differences between related genera is seldom possible because of sterility and lack of genetical or cytological information. A striking exception is provided by *Zea mays* L. and *Euchlaena mexicana* Schrad. Between these distinct species highly fertile hybrids are easily obtained. Genetic and cytological work with maize is sufficiently advanced to make analysis of hybrids profitable.

Linkage groups as well as cytological markers have been established for all 10 chromosomes in *Zea mays*. In some cases the approximate position of genes on a chromosome has been determined. Some of the most useful chromosome markers have been obtained as a result of the recent work with segmental interchange strains. The meiotic prophase crosses and rings which appear in maize heterozygous for an interchange make identification of the chromosomes concerned relatively easy.

A consideration of these facts led to the belief that hybrids of maize and annual teosinte would be exceptionally favorable material for cytogenetical studies. From the study of hybrids it was hoped that a clearer knowledge of the similarities and differences of the chromosomes of maize and its relative, annual teosinte, would be obtained. Knowledge of this kind is useful in estimating the evolutionary processes which have been effective in the differentiation of the two forms studied.

Three varieties of annual teosinte are recognized: Florida, Durango, and Chalco. The latter two varieties have been found growing wild in Mexico (Collins, 1921), whereas the cultivated Florida variety is thought to have come originally from Guatemala. Recently, Collins (1932) has reported that an annual teosinte resembling the Florida variety has been discovered which is native to that region.

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Morphologically, *Euchlaena mexicana* differs markedly from *Zea mays* especially in ear characters. BEADLE (1932) reports that the Florida variety is the only one which, when crossed with maize, produces noticeably sterile hybrids. The variety Durango, used to a lesser extent in our crosses, produces hybrids which appear to be almost completely fertile. A rather surprising fact reported by BEADLE (1932) is that hybrids between the Florida and Durango varieties are highly sterile, about 67 percent of the pollen grains being deficient in starch. In this case intervarietal sterility is greater than the inter-generic sterility found when either of these varieties is crossed with maize.

All 3 varieties of *E. mexicana* have the same chromosome number ($n = 10$) as has *Zea mays*. The morphological features of the chromosomes of the Florida variety are similar to those of maize (BEADLE, 1932), except for the presence of terminal knobs on 8 of the 10 chromosomes. As in maize, a satellite chromosome is present.

MATERIALS AND METHODS

To test the pairing relations of particular maize chromosomes with corresponding teosinte chromosomes, hybrids were obtained between teosinte and maize strains homozygous for known reciprocal translocations. A descriptive terminology introduced by ANDERSON (1935) is now used in denoting the various reciprocal translocations in maize. Thus, T1-2a is homozygous for the reciprocal translocation between chromosomes 1 and 2 which was first described. T1-2b would apply to a second translocation involving the same 2 chromosomes. Ordinary maize will be referred to in this paper by the old term *o*-normal to distinguish it from translocation types.

MCCLINTOCK (1930), COOPER and BRINK (1931) and others have shown that in maize heterozygous for an interchange involving 2 chromosomes, the 2 interchange chromosomes and the 2 "normal" ones with which they pair form a characteristic cross-shaped figure at pachytene stages of the first meiotic division. The cross complex opens out at diakinesis into a ring of 4 chromosomes. The formation of these cross-shaped complexes has added to the proof that prophase pairing of chromosomes is determined by homology of the pairing strands.

It was expected that structural differences between maize chromosomes and their respective teosinte "homologs" in hybrids would be revealed by modification of the structure of the cross-shaped complex, and by reduction in number or absence of chromosome rings at diakinesis and equatorial plate stages. In each such hybrid, pairing of both ends of 2 or more chromosomes is tested simultaneously. A list of the translocation stocks of maize used is given in table 1.

Anthers were fixed in various modifications of Carnoy's solution. COOPER's (COOPER and BRINK 1931) modification (2 parts chloroform: 2 parts glacial acetic:6 parts absolute or 95 percent alcohol) gave satisfactory results. After fixation for 2–10 hours, the material was washed in two changes of 95 percent alcohol and preserved in 80 percent or 70 percent alcohol. The best smear preparations were obtained from anthers freshly placed in 80 percent or 70 percent alcohol.

TABLE 1

Translocation stocks of maize used in crosses with teosinte.

MAIZE STOCK NEW TERMINOLOGY	CHROMOSOMES INVOLVED IN TRANSLOCATION	DESCRIBED BY
T1-2a	1 and 2	Brink 1927. Cooper and Brink 1931.
T1-6a	1 and 6	Cooper and Brink 1931.
T1-2aT1-6a	1, 2 and 6	Brink and Cooper 1932.
T1-7a	1 and 7	Burnham 1930.
T5-7a	5 and 7	from Anderson—not described.
T4-8	4 and 8	from Anderson—not described.
T8-9a	8 and 9	Burnham 1930. McClintock 1930, 1931. Creighton and McClintock 1931.
T1-2aT8-9a	1 and 2 8 and 9	Burnham 1930.

For the smear preparations, iron-aceto-carmine of almost full strength was used. Occasionally dilution by the addition of a little 45 percent glacial acetic was found desirable. Slight pressure was applied to the cover slip and the slide gently heated several times over an alcohol lamp as recommended by MCCLINTOCK (1930).

Crosses between linkage-tester stocks of maize and Florida and Durango teosinte were made to determine crossover values in the hybrids. The F_1 hybrids were backcrossed to the maize parent type in most cases and crossover percentages determined from the character of the backcross generation.

At the latitude of Madison, Wisconsin (43°), the Florida and Durango varieties of teosinte do not flower under field conditions until late September or early October. Plants from vernalized seeds, treated as recommended for maize by LYSSENKO (see WHYTE and HUDSON 1933) did not flower appreciably earlier than untreated plants. Short-day treatment as described by EMERSON (1924), begun May 15 on plants 1 month old was effective, however, in hastening the flowering period. Both Florida and Durango teosinte so treated began shedding pollen in the first week of August. However, this was not early enough to allow all the desired crosses to be made.

CYTOLOGICAL OBSERVATIONS ON F_1 HYBRIDS BETWEEN MAIZE AND ANNUAL TEOSINTE OF THE VARIETIES FLORIDA AND DURANGO

O-normal maize × Florida teosinte

No lack of pairing could be detected in the late pachytene stages. At diakinesis and at equatorial plate stages (plate 1A) an unequal open, or end-to-end, pair (a, a′), sometimes appearing as 2 univalents, could be distinguished in slightly over 50 percent of the cells (table 5). A somewhat larger unequal pair was detected in 20 percent of the cells.

In describing microsporocytes from similar hybrids BEADLE (1932) found diakinesis pairing regular except for the presence of 2 unequal univalents in about 50 percent of the cells examined. Less frequently he observed 4 univalents. Our results are in agreement. Two heteromorphic pairs are present in maize-Florida hybrids. The members of these pairs fail to remain attached, or are attached only at one end, in an appreciable proportion of the microsporocytes at diakinesis and equatorial plate stages.

O-normal maize × Durango teosinte

In the relatively few figures examined, no univalents were found. One or 2 end-to-end pairs, perhaps indicating a somewhat reduced chiasma frequency for those pairs, were present in about 25 percent of the cells examined (plate 1B, a, b).

Chromosome 6 of maize, in maize-teosinte hybrids

The sixth chromosome of maize can be recognized in a high proportion of meiotic prophase figures by the satellite at one end by which the chromosome is attached (or appressed) to the nucleole. In hybrids of maize with Florida teosinte pairing of the sixth maize chromosome with a corresponding teosinte chromosome is complete. Attempts were made to count the number of chiasmata present. The counts given in table 2 were made from diakinesis stages. Chiasmata were found to be not confined to any restricted region of the chromosome. If chiasmata are related to genetic crossing over, either as cause or effect, then in hybrids of Florida teosinte and maize, crossing over in this chromosome should be a rather common phenomenon.

Relatively few counts have been made on chiasma frequency in the sixth chromosome in maize-Durango hybrids. As with Florida hybrids, however, the modal number appears to be 2. The figures given here for chiasma frequency may be too low, since in the case of interstitial chiasmata it is often impossible to determine whether more than 1 is present. The lower figure was recorded in such doubtful cases. A size difference, if there is any, could not be established as existing between the sixth chromosome

EXPLANATION OF PLATE 1

The figures were drawn with the exception of M with a Spencer compens 20× ocular and a 2 mm Leitz achromatic objective N.A. 1.32, at table level with the aid of an Abbe camera lucida. With a tube length of 160 mm a magnification of about 2720× was obtained. The figures have been reduced two-thirds in reproduction.

A: O-normal maize×Florida teosinte. Equatorial plate stage. 9 bivalents and 2 unequal univalents (a, a′).

B: O-normal maize×Durango teosinte. Diakinesis stage. 10 pairs of chromosomes. 2 pairs end-to-end (a, b).

C: T1–2a maize×Florida teosinte. Diakinesis stage. T1–2a ring of 4 chromosomes, 7 pairs and 2 unequal univalents (a, a′). The components of one of the end-to-end pairs (b) appear to be unequal in size.

D: T1–2a maize×Florida teosinte. Equatorial plate. Ring of 4, 7 pairs and 2 unequal univalents (a, a′).

E, F, G: T1–2aT1–6a maize×Florida teosinte.

E: Diakinesis. Ring of 6 chromosomes and 7 pairs, including 2 heteromorphic pairs, a and b.

F: Equatorial plate stage. Ring of 6 chromosomes and 7 pairs, including two heteromorphic pairs, a and b.

G: Diakinesis. Ring of 6 drawn from 4 different cells.

H, I: T1–7a×Florida teosinte.

H: Diakinesis stage. Ring of 4 chromosomes (c), and 8 pairs. One open heteromorphic pair (b); and one end-to-end unequal pair (a).

I: Diakinesis stages. Rings drawn from 4 different cells. Lower, (d), open ring. The other 3 rings (a, b, c) are representative examples of the most frequently occurring ring type.

J: T5–7a×Florida teosinte. Diakinesis stage. Chain of 4 chromosomes (b), 7 pairs and 2 unequal univalents (a, a′).

K: T1–2aT8–9a maize×Florida teosinte. Diakinesis. T1–2a ring (c), T8–9a ring (b) and 6 pairs. 1 pair open, unequal (a).

L: T8–9a maize×Florida teosinte. Diakinesis. T8–9a ring (c) and 8 pairs. One pair end-to-end (a); 1 open, heteromorphic pair (b).

M, N, O: T1–2aT8–9a maize×Durango teosinte.

M: Diakinesis. T1–2a ring (a); T8–9a ring (b); and 6 pairs. ×1575.

N: Diakinesis. Typical T1–2a and T8–9a configurations are shown in this cell. T1–2a ring of 4 chromosomes (c). T8–9a as 2 end-to-end pairs (a, b). 6 other pairs of chromosomes.

O: Diakinesis stages: T8–9a configurations. (a) ring, (b) loose chain, (c) 2 "pairs," the most frequently observed type of association.

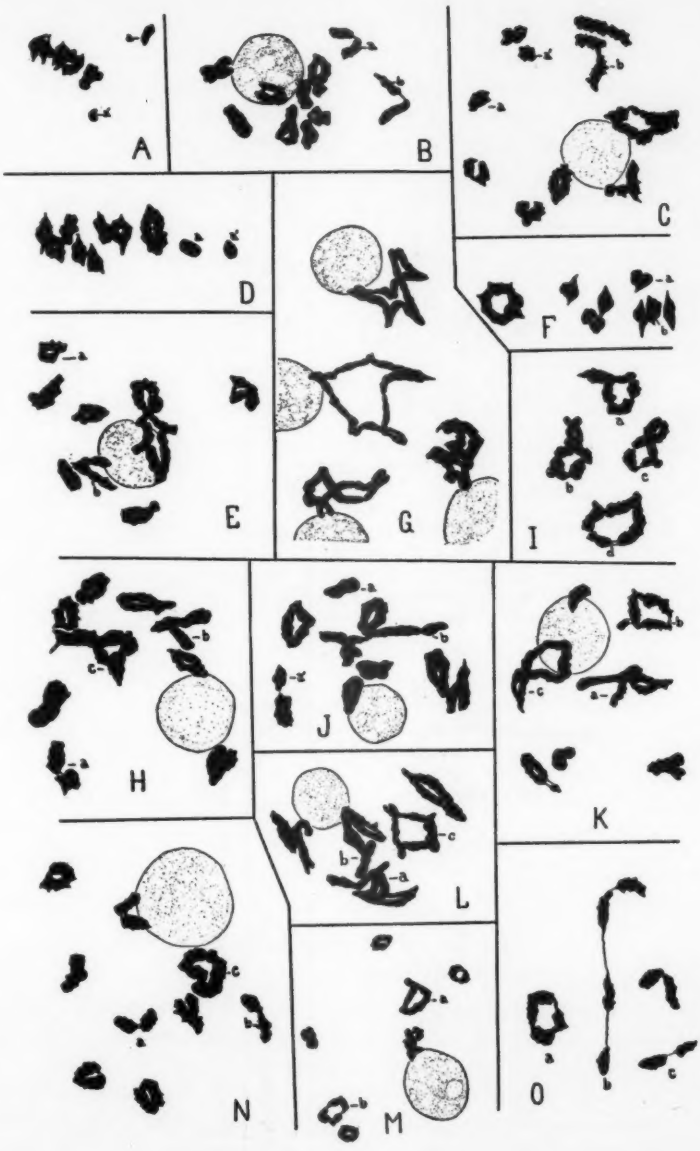
A
B
C
D
F
E
G
I
H
J
K
L
N
M
O

TABLE 2

Distribution of chiasmata in the 6th chromosome in F_1 hybrids of maize and teosinte (diakinesis figures).

	NO. OF PLANTS	0 CHIASMA	1 CHIASMA			2 CHIASMATA				3 CHIMASMATA				TOTAL CELLS
			PROX.	INTER	DISTAL	PROX. AND DISTAL	PROX. AND INTER	DISTAL AND INTER	2 INTER	PROX. INTER AND DISTAL	PROX. AND 2 INTER	DISTAL 2 INTER	3 INTER	
Maize×Florida	10	1	4	9	5	22	7	22	2	15	0	3	1	91
Maize×Durango	2	0	1	2	13	27	3	3	1	1	0	0	0	51

[1] Proximal refers to the satellite end of the chromosome.

of maize and the corresponding chromosome of either Florida or Durango teosinte. No lack of pairing could be observed.

T1–2a maize×Florida teosinte

The *P-br* and *B-lg* chromosomes, the 2 longest of maize, have exchanged segments in the T1–2a strain. Prophase crosses which were usually not marked by any striking peculiarities were observed in the microsporocytes of the hybrids. At diakinesis and at the equatorial plate stages chromosome rings (plate 1C, D) were found in 90.2 percent of the cells counted (table 4).

Both unequal pairs were to be seen in figures in which the ring was present (plate 1C, a, b). The cytological evidence indicates then that the first and second chromosomes of maize pair closely with their Florida "homolog," and that neither chromosome 1 nor 2 is involved in either of the 2 heteromorphic pairs.

TABLE 3

Diakinesis configurations of the interchange complex in T1–7a maize×Floirda teosinte hybrids.

CONFIGURATION	DESCRIPTION	DIAGRAM	NUMBER
ring	1 or more interstitial chiasmata in long chromosomes		73
ring	1 interstitial chiasma in long, 1 interstitial chiasma in short chromosomes		3
ring	"distal" ends of long chromosomes free but one interstitial chiasma		6
ring	open-chromosomes held together only at the ends		
chain			29
			7
		Total	118

T1–6a maize×Florida teosinte
T1–2aT1–6a maize×Florida teosinte

Few cells of T1–6a maize×Florida teosinte hybrids were examined. Diakinesis rings of 4 chromosomes appeared to be common, the ring being attached, at early stages, to the nucleole.

BRINK and COOPER (1932) showed that in the T1–2aT1–6a strain chromosome 1 has exchanged different segments with chromosomes 2 and 6. As would be expected from the previously recorded observations on

pairing of chromosomes 1, 2, and 6 of maize in hybrids with Florida teosinte, the predominant diakinesis configuration was a ring of 6 chromosomes (plate 1G) and 7 pairs (plate 1E, F). Since both heteromorphic pairs (a, b) could still be distinguished the chromosomes involved in them are none of those in the T1–2a,T1–6a ring of 6. The high proportion of diakinesis rings, 88 percent (table 4), provides further evidence of intimate pairing of chromosomes 1, 2, and 6 of maize with the corresponding chromosomes of Florida teosinte.

T1–7a maize×Florida teosinte

The strain of maize designated as x-normal-3 by Burnham (1930) is homozygous for a reciprocal translocation between the first and seventh chromosomes. Prophase crosses involving the 2 interchange chromosomes of maize and 2 from the teosinte parent were not found in the few pachytene stages observed in microsporocytes of the hybrids. Diakinesis rings (plate 1H, c, I) were present in 94 percent (table 4) of the cells examined at those stages. A large proportion of the rings (plate 1I, a, b, c) were of a form which indicated that one or more interstitial chiasmata were present in the 2 long chromosomes. Counts of the different types of rings were made (table 3) to determine the approximate proportion of cells having the semi-closed, or figure-8 type of ring.

Since 62 percent of the microsporocytes had chromosome rings of the general type described, pollen counts were made to determine whether an effect on fertility could be discerned. Such an effect might be expected if the prevailing ring configuration affected anaphase distribution of the ring chromosomes, favoring either the passage of alternate or adjacent chromosomes to the same pole. Over 60 percent of the pollen grains examined were deficient in starch, indicating that fertility, if changed at all, is lowered rather than raised by the predominant ring type present at the diakinesis stages in the microsporocytes of these hybrids.

Both unequal pairs of chromosomes (plate 1H, a, b) were also distinguishable in diakinesis figures of these hybrids (table 5) indicating that the seventh maize chromosome is not involved in either pair.

T5–7a maize×Florida teosinte

The fifth and seventh maize chromosomes have exchanged segments in the T5–7a stock. Microsporocytes from only 1 hybrid plant have been obtained as yet. Diakinesis rings consisting of 4 chromosomes were present in 73 percent of the diakinesis figures examined. The larger, less-frequently observable unequal pair was not distinguishable in any of the cells but the smaller, more markedly unequal pair was noted (figure 1 and plate 1J, a, a′) in a number of instances. Too little material has been examined,

TABLE 4

The number and proportions of the diakinesis translocation configurations found in maize-teosinte hybrids.

HYBRID	MAIZE TRANSLOCATION CHROMOSOMES	RING		CHAIN		2 PAIRS		TRIVALENT AND UNIVALENT		PAIR AND 2 UNIVALENTS		OTHER CONFIGURATIONS		TOTAL
		NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	
T1-2a×Florida	1 and 2	55	90.2	5	8.2	1	1.6							61
T1-6a×Florida	1 and 6											chain of 4 and 1 pair		
T1-2aT1-6a×Florida	1, 2 and 6	164	88.2	21	11.3							1	0.5	186
T1-7a×Florida	1 and 7	111	94.1	7	5.9									118
T5-7a×Florida	5 and 7	37	72.6	7	13.7	7	13.7							51
T4-8×Florida	4 and 8	5	15.6	18	56.2	2	6.3	7	21.9					32
T8-9a×Florida	8 and 9	36	53.7	17	25.4	13	19.4			1	1.5			67
T1-2aT8-9a×Florida	1 and 2	207	89.2	20	8.6	5	2.2							232
	8 and 9	118	50.9	61	26.3	34	14.7	8	3.4	11	4.7			232
T1-2aT8-9a×Durango	1 and 2	97	92.4	6	5.7	2	1.9							105
	8 and 9	7	6.7	12	11.4	77	7.33	1	1.0	8	7.6			105

however, to be conclusive. The meager data suggest that in this hybrid ring formation is somewhat reduced, and that the larger of the 2 heteromorphic pairs may be included in the translocation complex in the hybrid.

T4–8 maize×Florida teosinte

The interchange complex in microsporocytes of T8–9a maize×Florida teosinte hybrids frequently takes the form of 2 "pairs." It is consequently of interest that in T4–8 maize×Florida teosinte hybrids a chain of 4 is most frequently observed. Other configurations occur (table 4), though

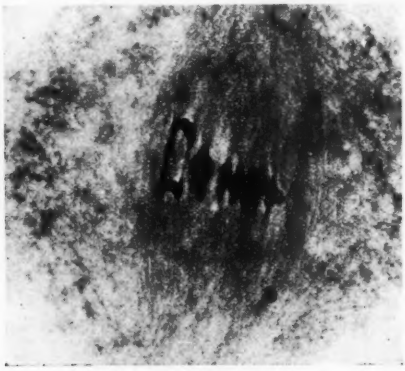

FIGURE 1.—T5–7a maize×Florida teosinte. Equatorial plate stage. Ring of 4 chromosomes, 7 pairs, and 2 unequal chromosomes. The unpaired chromosomes are off the plate. Photomicrograph. 850×.

less frequently. The appearance of the T4–8 complex has not been figured in the literature, but presumably rings of 4 are most common. It is suggested that a segment of the 8th maize chromosome frequently fails to be attached by a chiasma to a corresponding teosinte chromosome segment. The smaller unequal pair of chromosomes was noted in about 30 percent of the microsporocytes (table 5).

T8–9a maize×Florida teosinte
T1–2aT8–9a maize×Florida teosinte
T1–2aT8–9a maize×Durango teosinte

The point at which the translocation break has occurred in the ninth chromosome in T8–9a maize has been established by a combination of cytological and genetical methods (BURNHAM 1930; MCCLINTOCK 1931; CREIGHTON and MCCLINTOCK 1931). In the T1–2aT8–9a strain of maize the 2 translocation chromosomes of the T1–2a stock are also present. The

TABLE 5

Numbers and proportions of heteromorphic pairs of chromosomes in diakinesis figures of maize-Florida teosinte hybrids.

HYBRID	NUMBER OF PLANTS	SMALL UNEQUAL		MEDIUM UNEQUAL		TOTAL CELLS	PERCENT SMALL UNEQUAL	PERCENT MEDIUM UNEQUAL
		2 UNIVALENTS	"PAIR"	2 UNIVALENTS	"PAIR"			
0-normal maize ×Florida teosinte	7	44	41	7	23	150	56.7	20.0
T1-2a maize ×Florida teosinte	2	21	5	2	9	61	42.6	18.0
T1-2aT1-6a maize ×Florida toesinte	2	23	36	2	32	186	31.7	18.3
T1-7a maize ×Florida teosinte	2	11	3	1	3	31	45.1	12.9
T5-7a maize ×Florida teosinte	2	6	12	0	0	51	35.3	—
T4-8 maize ×Florida teosinte	2	3	7		2	32	31.2	6.2
T8-9a maize ×Florida teosinte	4	?	?	0	8	67	—	11.9
T1-2aT8-9a maize ×Florida teosinte	5			7	30	180	—	20.6

T8–9a interchange complex in hybrids of these translocation stocks by Florida teosinte takes the form of a ring at diakinesis (plate 1K, b, L, c) in approximately one-half of the cells counted. A chain of 4, 2 "pairs," a trivalent and one univalent, and a pair and 2 univalents were the other observed configurations (table 4). At least 1 of the heteromorphic pairs (plate 1K, a) was also present (table 5). In the T1–2aT8–9a maize×Durango teosinte hybrids the T1–2a ring (plate 1M, a, N, c) was present at diakinesis in over 90 percent of the cells examined; the T8–9a ring (plate 1M, b, O, a) in only 6.7 percent (table 4).

In both Durango and Florida teosinte hybrids with T8–9a, the interchange complex, at diakinesis, takes the form of a ring of four chromosomes much less frequently than it does at corresponding stages in pure maize heterozygous for the translocation, that is, 97 percent according to BEADLE (1932). If ring formation depends on the formation of 1 or more chiasmata in each arm of the prophase cross, then the very marked reduction in number of rings formed in these hybrids indicates failure of chiasma formation in 1 or more arms of the pachytene cross in a rather high proportion of cases.

SUMMARY OF CYTOLOGICAL RESULTS

A summary of the general results is presented in tables 4 and 5. The ailable cytological evidence indicates close correspondence of chromosomes 1, 2, 6, and 7, somewhat less for chromosome 5, of maize with Florida teosinte chromosomes. The reduced number of rings in T8–9a maize×Florida teosinte hybrids suggests that a structural difference preventing chiasma formation exists between the eighth or ninth chromosomes, or both, of maize and the Florida teosinte chromosomes with which they pair, at least partially. Even less correspondence of these chromosomes in Durango hybrids is indicated. Cytological evidence of approximately normal pairing between chromosomes 1, 2 and 6 of maize with Durango teosinte chromosomes has been obtained.

CYTOGENETIC STUDIES INVOLVING THE NINTH MAIZE CHROMOSOME IN MAIZE-TEOSINTE HYBRIDS

The genes used, together with their position in the linkage group as given by EMERSON (1932) are listed below.

yg₂—yellow green seedling and plant; at locus 0
sh —shrunken endosperm ; at locus 22
wx —waxy endosperm ; at locus 52

The spindle attachment region is believed to lie near the *wx* locus. The translocation point lies some 12 genetic units from *wx* on the long arm of

TABLE 6

Backcross data on crossing over between chromosome 9 of maize and the corresponding chromosome of Florida teosinte.

INVESTIGATOR	TEOSINTE	Y_{Gr}-*Sh*	Y_{Gr}-*sh*	y_{Gr}-*Sh*	y_{Gr}-*sh*	*Sh*-*Wx*	*Sh*-*wx*	*sh*-*Wx*	*sh*-*wx*	TOTAL
Beadle	Florida	551	3 0.3%	0 0.0%	475	550	1 0.1%	0 0.0%	478	1029
Arnason	Florida	569	14 1.5%	10 1.0%	366					959
Arnason	Florida					978	42 2.0%	9 0.4%	1031	2060

the chromosome (BEADLE 1932). No genes are known in the long arm.

yg₂ sh wx maize was crossed with Florida teosinte, which carries the corresponding dominant allelomorphs. The hybrids were then backcrossed to the recessive parent. The backcross seeds and plants were classified with the results as given in table 6, where they are compared with those of BEADLE (1932).

Because of poor germination of backcross seeds, more than twice as many seeds were classified for the endosperm characters *sh* and *wx* as could be classified for the plant character yg_2. The results of the two independent investigations summarized in table 6 are in agreement to the extent that both show crossing over to be much less frequent in the short arm of the ninth chromosome in maize-Florida teosinte hybrids than in maize.

A comparison of Beadle's results with those of the writer reveals that the crossover values obtained by the latter are higher for both yg_2–*sh* and *sh*–*wx* regions of the ninth chromosome.

The plants classified as $\frac{Yg_2\text{–}sh\text{–}wx}{yg_2\text{–}sh\text{–}wx}$ or $\frac{yg_2\text{–}Sh\text{–}Wx}{yg_2\text{–}sh\text{–}wx}$ were grown to maturity to check their classification. Ten authentic crossovers were established. The remainder proved to be non-crossovers and may have been the result of hetero-fertilization (SPRAGUE, 1932). Plants from the seeds classified as non-shrunken waxy, or shrunken, non-waxy will also be grown. Seeds of these 2 types cannot be accounted for on the basis of hetero-fertilization, and as contamination is rather unlikely, crossing over in the F_1 hybrids is the most probable reason for their occurrence.

The difference between the crossover values reported here and those obtained by BEADLE may be due to differences in the Florida teosinte plants used as parents, or they may be due to environmental or genic differences affecting the rate of crossing over (STADLER 1925, 1926). The fact that BEADLE used third and fourth generation backcross plants may have had some effect, although the crossover values presented by EMERSON and BEADLE (1932) for successive backcross generations are uniformly low in all.

If chiasmata formed in meiotic prophase condition diakinesis and equatorial plate association of the members of a bivalent (DARLINGTON 1932) then a test of the assumption that every chiasma represents a crossover can be proposed here. In maize heterozygous for the T8–9a translocation one arm of the pachytene cross-shaped configuration consists of the short arm of the ninth chromosome and approximately 12 genetic map units besides (MCCLINTOCK 1931). BEADLE (1932) finds that crossing over in the *wx*-translocation region is approximately the same in maize-Florida teosinte hybrids as in maize, that is, 12 percent, but in all the short arm

TABLE 7

Interchange configurations in hybrids of T8-9a maize×teosinte.

INVESTIGATOR	TEOSINTE PARENT	MAIZE PARENT	NO. OF PLANTS STUDIED	CHROMOSOMES IN COMPLEX	RING OF 4	CHAIN OF 4	2 "PAIRS"	TRIVALENT AND UNIVAL.	PAIR AND 2 UNIVALENTS	TOTAL	PERCENT RINGS
Beadle	Florida	T8-9a	5	8 and 9	16	62	158	6	2	244	6.46
Arnason	Florida	T8-9a	4	8 and 9	36	17	13	0	1	67	53.7
Arnason	Florida	T1-2a-T8-9a	5	8 and 9	118	61	34	8	11	232	50.9
Beadle	Durango	T8-9a	4	8 and 9	46	183	4	4	0	237	19.0
Arnason	Durango	T1-2a-T8-9a	3	8 and 9	7	12	77	1	8	105	6.7

(52 map units in maize) only 5 percent of crossing over, or less, occurs in the hybrids. One complete arm of the translocation prophase cross is accounted for by the genetic data. A total of 17 percent of crossing over occurs in that whole arm. On BELLING's theory, with 17 percent of crossing over, there should be one chiasma present in that arm in only 34 percent of the cells. At diakinesis the 66 percent of cells without a chiasma in that arm should show the chromosomes of the T8–9a complex in configurations other than a ring. The exact form taken will depend on the pairing relations of most of the long arm of chromosome 9 and all of chromosome 8 with the teosinte chromosomes in the complex. Thirty-four percent of microsporocytes might be expected to show a chromosome ring configuration at diakinesis if chiasmata are regularly formed in the other 3 arms of the complex. Our counts on diakinesis configurations, reported in the previous section, gave the proportion of cells having a ring of 4 chromosomes at diakinesis as approximately 50 percent. BEADLE (1932) (table 7), however, gives a much lower figure.

On the assumption that often no chiasma is formed in one arm of the eighth chromosome, the low percentage (6.46) of diakinesis rings of 4 chromosomes in the T8–9a maize × Florida teosinte hybrids might appear to be in accord with BELLING's theory. This is the more plausible when the high frequency of 2 end-to-end pairs is noted. The formation of a chiasma in the yg_2–T region of the ninth chromosome may not, on that account, always insure ring formation. The figures here reported for the first time cannot be explained on that basis. Either too many cells in the doubtful, uncounted class had configurations other than rings, or else the actual number of rings obtained is higher than would be expected on BELLING's theory. Some of the material had been stored in 70 percent alcohol for some months and was not ideal for critical examination of the T8–9a configuration. Satisfactory preparations, however, were obtained.

No backcross data are available with regard to crossing over in the yg_2–sh–wx–T region of the ninth maize chromosome in hybrids with Durango teosinte, except those reported by BEADLE (1932). He found complete absence of crossing over in the yg_2–sh–wx region, but the percentage of crossing over in the wx–T region was about the same as in pure maize, namely, 12 percent. The proportion of diakinesis rings reported by him (19 percent) agrees fairly well with what would be expected on BELLING's theory. In our material, however, the proportion of rings, and chains as well, was much lower, the predominant configuration being 2 end-to-end pairs (plate 1N, a, b, O, c). The high proportion of 2 end-to-end pairs (table 4) suggests that one arm of the eighth chromosome of maize, as well as one of the ninth, often fails to have a chiasma attachment to a Durango teosinte "homolog." Unfortunately, no genetic tests of crossing

TABLE 8
Crossing over in Maize-Florida teosinte hybrids. Backcross data.

MAIZE CHROMOSOME	CONSTITUTION OF HYBRID	NUMBER OF INDIVIDUALS					CROSSOVERS		MAP DISTANCE[1] IN MAIZE
		XY	*Xy*	*xY*	*xy*	TOTAL	NO.	PERCENT	
1	$\frac{br\ f}{+\ +}$	1		2*	3	6	2	33.3	6
	$\frac{f\ bm_2}{+\ +}$	2	1	2*	1	6	3	50.0	64
	$\frac{br\ bm_2}{+\ +}$		1	4	1	6	5	83.3	70
2	$\frac{v_4 gl_2}{+\ +}$	126	114	85	184	509	199	39.1	51
	$\frac{gl_2 g_1}{+\ +}$	169	42	95	203	509	137	26.9	20
	$\frac{v_4 g_1}{+\ +}$	140	100	124	145	509	224	44.0	71
3	$\frac{lg_2 d_1}{+\ +}$	12	8	6	15	41	14	34.1	30
7	$\frac{gl_1 v_6}{+\ +}$	72	3	6	95	176	9	5.1	6

[1] Data from EMERSON (14).

* No sign of fine stripes but the classification was not checked by backcrossing to *f* stock.

over in the eighth chromosome of maize in maize-Durango or maize-Florida teosinte hybrids have been reported. Only 2 genes have been located in the eighth chromosome. Adequate tests of crossing over, therefore, are impossible.

CROSSING OVER BETWEEN OTHER CHROMOSOMES IN MAIZE-FLORIDA TEOSINTE HYBRIDS

F_1 plants obtained by crossing linkage tester stocks of maize with Florida teosinte were backcrossed to the maize lines. Genetic tests involving regions of chromosomes 1, 2, 3 and 7 have been made. The results are presented in table 8.

It is established that crossing over occurs between maize and Florida teosinte chromosomes in the regions which were under observation. Where the numbers are large enough to be indicative the crossover values appear to be not very different from those found in pure maize.

Genetic tests by EMERSON and BEADLE (1932) showed that crossing over in segments of chromosomes 2, 5 and 10 in maize-Florida teosinte hybrids was about as frequent as in maize. The short arm of chromosome 9 is the only chromosome segment in which crossing over has been shown to be markedly reduced in these hybrids.

DISCUSSION

The observations on microsporocytes of hybrids between Florida teosinte and strains of maize carrying known translocations has supplied more precise information regarding the degree to which pairing takes place between particular chromosomes of maize and the corresponding chromosomes of teosinte than can be had by observation on microsporocytes of ordinary maize by teosinte hybrids. If the chromosome ring configurations seen in diakinesis stages in plants heterozygous for a translocation owe their formation to previous chiasma formation in each arm of the pachytene cross-shaped structure, then the high proportion of rings seen in hybrids of T1–2a, T1–2aT1–6a, T1–7a with Florida teosinte provides proof that chiasmata are regularly formed at or near both ends of the first, second, sixth and seventh chromosomes. This may be used as evidence that very considerable portions of these maize chromosomes have their structural counterparts in chromosomes of Florida teosinte. Pairing of chromatids at pachytene stages has been shown by various workers to be determined by homology of the pairing segments. Especially instructive are the cross-shaped configurations seen at pachytene stages in microsporocytes of maize heterozygous for reciprocal translocation. These configurations are interpretable on the grounds that chromatids lie in close association where they are homologous; that where homology ends, pairing likewise ends (MCCLINTOCK 1930; COOPER and BRINK 1931). BURNHAM (1931) and MCCLINTOCK (1932), however, reported close association

of short non-homologous maize chromosome segments. If pachytene pairing of parts of non-homologous chromatids occurs, and chiasmata are not formed, diakinesis association of chromosomes should be a more reliable indication of correspondence than earlier prophase figures. Should chiasmata occur in the paired regions, translocation, reduplication and deficiency would be expected. The effects would presumably be reflected in gametophyte sterility or aberrant genetic ratios. Intimate association of non-homologous chromosome segments combined with chiasma formation must be rare in maize since the resulting unequal crossing over would be readily detected.

Parts of the fourth, fifth, eighth and ninth chromosomes of maize appear to conjugate with parts of corresponding Florida teosinte chromosomes. The extent of correspondence of the fifth and eighth chromosomes with Florida teosinte "homologs" has not been fully determined but is suspected of being incomplete. The short arm of the ninth maize chromosome almost certainly does not correspond with a part of the teosinte "homolog."

Tests of crossing over in maize-Florida teosinte hybrids have been made for segments of 7 chromosomes by EMERSON and BEADLE (1932) and the writer. In 6 of the chromosomes crossing over appears similar in amount to that in maize. The genetic as well as the cytological evidence indicates that the chromosomes of maize and Florida teosinte correspond closely but that the correspondence is not complete for all the chromosomes.

The results suggest that gene changes rather than changes in the gross structure of the chromosomes may have been chiefly responsible for the differentiation of maize and annual teosinte.

SUMMARY

1. Pairing of chromosomes 1, 2, 6 and 7 of maize and corresponding Florida teosinte chromosomes appears to be complete in hybrids.

2. Relatively long segments of either the eighth or ninth maize chromosome frequently fail to form chiasmata with teosinte "homologs."

3. Only 5 percent of crossing over occurs in maize-Florida teosinte hybrids in a segment of the ninth maize chromosome which includes 52 map units.

4. Some cytological evidence of possible slight structural difference between chromosome 5 of maize and the Florida teosinte chromosome with which it pairs, at least in part, is presented.

5. Crossing over occurs between genes in maize chromosomes 1, 2, 3 and 7 and Florida teosinte chromosomes. The frequency of crossing over in hybrids was not accurately determined but the few data obtained suggest that the values are not very different from those in pure maize.

6. Conclusive evidence is not yet available as to which maize chromo-

somes are members of the two heteromorphic pairs found in microsporocytes of maize-Florida teosinte hybrids. Evidence that chromosomes 1, 2, 6 and 7 are not involved has been obtained. Chromosome 5 may be a member of one of the unequal pairs, the other is probably chromosome 8, 9 or 10.

7. Pairing of chromosomes 1, 2 and 6 of maize with Durango teosinte chromosomes appears to be complete.

8. Cytological evidence suggests that chiasmata are very rarely formed between relatively long segments of both chromosome 8 and 9 of maize with corresponding Durango teosinte chromosomes.

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A MORPHOLOGICAL COMPARISON OF TRIPLOID AND TETRAPLOID INTERSPECIFIC HYBRIDS IN TRADESCANTIA

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ONE of the fundamental methods of genetic investigation has been to vary the balance between selected elements in the germplasm and compare the resulting combinations. Outstanding examples of the successful application of this method are BRIDGE'S (1925) studies of the balance between the sex chromosome and the autosomes and MULLER'S (1931) classification of mutant factors by a comparison of their effects in disomic and trisomic combinations. The American species of Tradescantia, several of which exist in both diploid and auto-tetraploid races (ANDERSON and WOODSON 1935; ANDERSON and SAX, in press) make it possible to apply the same method to the study of specific differences. The following paper reports the results of crosses between a common tetraploid species, *Tradescantia subaspera* Ker-Gawl var. *typica* ANDERSON and WOODSON (*T. pilosa* Lehm.) and diploid and tetraploid races of *T. canaliculata* Raf. (*T. reflexa* Raf.).

THEORETICAL

This experiment provides us with four forms for comparison, the two species, the triploid hybrid, and the tetraploid hybrid. The diploid and tetraploid races of *T. canaliculata* are morphologically indistinguishable, as is generally the case among these American Tradescantias (ANDERSON and SAX, in press). Using the letter S to represent a genom of *T. subaspera* and the letter C to represent a genom of *T. canaliculata*, we may diagram the first species as SSSS, the latter as CCCC (or CC in the case of the diploid race). The two possible hybrids we can denote as CSS and CCSS, thereby emphasizing the fact that the former has two sets of chromosomes from *T. subaspera* but only one from *T. canaliculata*, whereas in the latter hybrid the two species have each contributed two sets.

Assuming that the differences between the two species are genic, or largely so, what differences may we expect between the two hybrids? Let us first of all examine the consequences to be expected if the species are differentiated by dominant genes, dominants and recessives being distributed about equally to both species. In the case of a dominant contributed by *T. subaspera*, the formulae for the two hybrids will be AAa and AAaa. For a dominant gene the effects of these two combinations should be indistinguishable, or practically so. If the dominant is con-

tributed by *T. canaliculata*, the two hybrid formulae are Bbb and BBbb. In this case we shall ordinarily expect the two combinations to be practically indistinguishable, since if we define a dominant gene as one in which the combination AA has practically the same effect as Aa, we know from past experience that for the majority of such genes Aaa is similar in effect to Aa and hence to AAaa. In the hybrids, therefore, if the genes distinguishing the two species are dominant, or largely so, we may expect CCSS and CSS to be essentially similar. Since, in point of fact, the triploid hybrid CSS is strikingly different from the tetraploid hybrid CCSS we may conclude *that the essential differences between the two species, if genic, are largely without dominance.*

EXPERIMENTAL DATA

While the characters which distinguish the species of Tradescantia are not so precise, nor so unaffected by the environment, as one might wish,

TABLE 1

Comparison of triploid and tetraploid hybrids between T. canaliculata and T. subaspera var. typica

	T. canaliculata	TETRAPLOID HYBRID	TRIPLOID HYBRID	*T. subaspera* VAR. *typica*
	CCCC	*CCSS*	*CSS*	*SSSS*
Internode length	16 cm.	11 cm.	8.5 cm.	3 cm.
Leaf length ÷ width	23	16	10	7
Stomata on upper surface	none	few	several	many
Sepal length	1.1 cm.	0.9 cm.	0.8 cm.	0.7 cm.
Pubescence of pedicel	none	slight	medium	heavy

the two species chosen for the experiment differ as sharply as any two within the group related to *T. virginiana*. Unfortunately, *T. subaspera* var. *typica* is so far known only as an autotetraploid; were a diploid race to be discovered it would permit comparison of three different intermediate balances with the two pure species. The experiment is furthermore unfortunate in the fact that the only tetraploid *T. canaliculata* in flower at the time the crosses were made was a somewhat exceptional individual with slightly broader leaves and a larger number of nodes than is normal for the species. In both of these characteristics it departed from the normal in the direction of *T. subaspera* var. *typica*, thereby reducing slightly what might have been an even greater difference between the triploid and tetraploid hybrids. The node number of this tetraploid parent was so exceptionally high that this character, which would otherwise have been one of the best for comparison, has been omitted altogether. Both crosses were made with *T. canaliculata* as the female parent. For the triploid, a diploid plant from Austin, Texas, was pollinated from a plant of *T. sub-*

aspera var. *typica* collected at Marthasville, Missouri. The tetraploid had the same pollen parent, its seed parent being a tetraploid plant of *T. canaliculata* from Hamburg, Missouri.

Detailed comparisons for both hybrids with the parental species are made in table 1 and text-figure 1. The internodes used in the table and the figure were chosen from comparable points on all four plants, being the first internode below the lowest branch of the inflorescence and the leaf

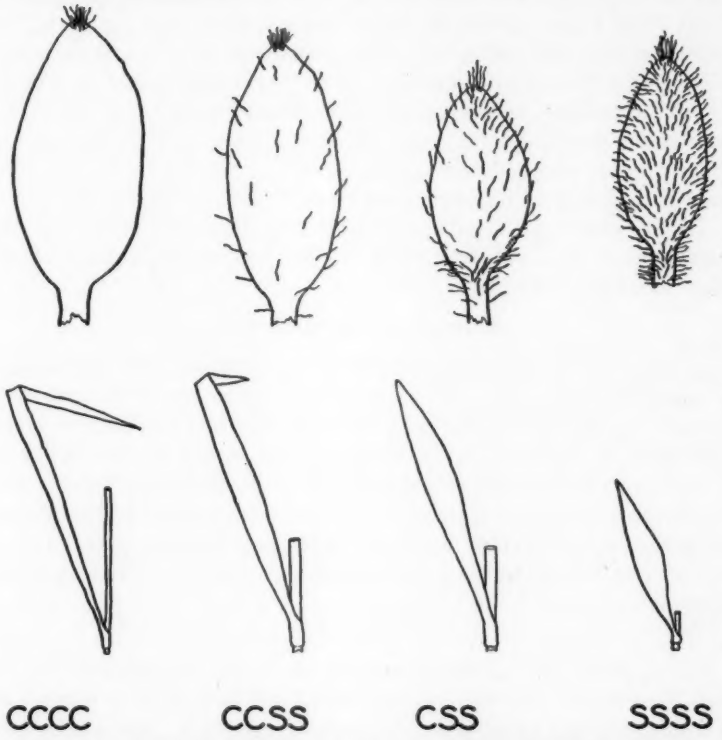

FIGURE 1.—Above: Calyx and pedicels (×10); Below: Leaf and internode (×1.3) of *T canaliculata* (CCCC), *T. subaspera* (SSSS), their triploid (CSS) and tetraploid (CCSS) hybrids. While the calyces have been carefully drawn to scale they are somewhat diagrammatic.

subtending this internode. It will be noted from figure 1 that this character is apparently somewhat affected by hybrid vigor. Leaf shape is probably the most reliable of the characters, since it is only slightly affected by hybrid vigor and by environmental influences. It will be seen that the hybrid CCSS is almost exactly intermediate between the two species in its

leaf index and that the triploid hybrid CSS is again almost exactly intermediate between it and SSSS.

The relative abundance of stomata on the upper and lower surfaces of the leaf is a character of much taxonomic importance in the genus Tradescantia (ANDERSON and WOODSON 1935). In *T. subaspera* var. *typica* there are virtually no stomata on the upper surface; in *T. canaliculata* they are about equally abundant on both surfaces. The two hybrids CSS and CCSS present two intermediate stages between these end points. Table 1 and figure 1 present a tabular and graphical summary of the five characters most susceptible to precise comparison. In each case the tetraploid hybrid CCSS is intermediate between the two species and the triploid CSS is intermediate between it and SSSS. Other less objective characters presented a similar result; young plants of CCSS, for instance, were in general aspect clearly intermediate between the two parents and were recognized as such by naturalists familiar with the two species. The plants of CSS, however, particularly in their younger stages, presented more the appearance of a puzzling variant of *T. subaspera*, to those unacquainted with the origin of the plants.

SUMMARY AND CONCLUSIONS

As measured by five unrelated characters, tetraploid hybrids between *T. subaspera* var. *typica* and *T. canaliculata* were exactly intermediate between the two species. By the same measure, triploid hybrids to whose germplasm *T. subaspera* var. *typica* had made twice the contribution of *T. canaliculata*, were intermediate between the tetraploid hybrids and *T. subaspera* var. *typica*. Following the argument developed in the theoretical section, it is concluded that in so far as the differences between these two species are genic, they rest largely upon genes with incomplete dominance.

NOTE

It is perhaps worth calling attention to the taxonomic significance of the facts reported in the above paper. Polyploidy introduces a number of complications into the relationships between species. In this case the two races of *T. canaliculata* are morphologically indistinguishable, yet outcrossed to another species, *T. subaspera*, they produce two sets of hybrids which are very dissimilar morphologically, one of which (the triploid) is almost completely sterile, and one of which (the tetraploid) is semi-fertile. The case illustrates one out of many possible ways in which crosses between the same two species can give different results under different conditions.

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STUDIES OF A SIZE CROSS IN MICE

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IT WILL be conceded generally by students of genetics that body size, though clearly subject to heredity, is not inherited in a simple Mendelian way.

When races of unlike body size are crossed, the offspring are, in general, intermediate in body size, both in the first and in later generations of offspring. Most investigators in this field, being convinced that Mendel's law is universally valid, have accepted what is known as the multiple factor interpretation of this and other similar cases of intermediate or blending inheritance. On this hypothesis the genetic basis of differences in body size consists exclusively of mutated genes borne in the chromosomes. It is certain that such genes exist. Specific evidence for their existence will be described in this paper. But we are not convinced that no cell structures other than chromosomes are concerned in the determination of body size for the following reasons.

Some years since, a study was made of the inheritance of body size in a cross between races of rabbits, one of which was about four times as large as the other. The blending character of the inheritance indicated that, on a multiple factor interpretation, differential genes influencing body size must be so numerous and widely distributed as to be found in practically all chromosome pairs. An attempt was accordingly made, by crossing a large race in which four chromosomes carried dominant color genes with a small race carrying the corresponding recessive genes, to ascertain whether these four chromosome pairs bore any of the hypothetical genes influencing body size. The result was wholly negative. No difference in size could be detected between segregates which bore one or all of the dominant genes received from the large race parent and those which bore recessive genes derived from the small race parent. Nevertheless by embryological studies, CASTLE and GREGORY were able to show that the eggs of large-race and small-race rabbits develop at a different rate. Though the eggs are of the same size, that of the large-race rabbit undergoes cleavage more rapidly and thus produces a larger embryo. The more rapid rate of growth persists after birth and even until adult size is attained. Moreover, sperm as well as egg is influential in determining the developmental rate of the zygote. It seems, accordingly, that developmental rate is itself either gene controlled or controlled by some other feature of the organization of both sperm and egg.

About the time that this conclusion had been reached in our rabbit studies, GREEN made a cross between two species (or subspecies) of mice (*Mus bactrianus* and *Mus musculus*), the former of which is only about half as large as the latter. He applied the same criterion which CASTLE had used in the rabbit size cross for possible linkage between color genes and size genes, but with better success. He reported association in F_2 and backcross populations of larger size with the recessive color genes brown, dilution, and non-agouti, which came from the larger parent in the original cross. He inferred correctly that this implied the location in the three chromosome pairs under consideration of genes influencing body size. Later, when larger numbers had been studied, he concluded that the differences reported were of significant magnitude in one only of the three cases—that of the chromosome carrying the gene for brown. On this alone he based the argument for a multiple factor interpretation of body-size inheritance, supporting it later by evidence thought to indicate crossing over between the brown gene and one or more size genes located in the same chromosome.

We shall show that GREEN was undoubtedly right in assigning an influence making for greater body-size to both the brown chromosome and the dilute chromosome. The case for the non-agouti chromosome is doubtful. But we shall show, also, that the plus influence on body-size exerted by the brown chromosome and the dilute chromosome is due not, probably, to some hypothetical size gene linked with the color gene, but to the physiological action of that gene itself.

Our original plan was to verify, if possible, GREEN'S findings by repeating his experiment with certain minor modifications. In this we had the cordial coöperation of Dr. GREEN, who supplied us with animals of the same inbred stocks which he had used in his experiment—namely, *M. bactrianus* and LITTLE'S well known and long inbred dilute brown strain, both from the JACKSON MEMORIAL LABORATORY. We owe hearty thanks, in this connection, to Director C. C. LITTLE, and Drs. GREEN and MURRAY.

In the first cross which we made, on which we shall report in this paper, we used as mothers, not LITTLE'S dilute brown race but a derivative of it established by GATES, in which pink-eye and short-ear had been added to the assemblage of recessive characters. This race accordingly was homozygous for the five recessive characters, pink-eye, dilution, short-ear, brown, and non-agouti. It had been closely inbred in brother-sister matings for several generations. Two of the five recessive genes, namely, dilution and short-ear, are borne in the same chromosome and presumably very close together, since the occurrence of crossovers is only about one in 1000. Accordingly, four chromosomes are in this race tagged with recessives,

namely, (1) brown, (2) dilution and short-ear, (3) pink-eye, and (4) non-agouti. For brevity, we shall refer to this race as the s.e. race. As the other parent race we used not wild *M. bactrianus*, as GREEN had done, but a supposedly domestic derivative of it, the long inbred strain of black-and-white Japanese waltzing mouse used by Gates (1926). This carries three independent recessive characters, piebald, waltzing, and non-agouti.

In weight 17 adult males of the s.e. race range from 22 to 33 grams, average 26.2. Gates (1926) reports the average weight of adult Japanese waltzing males to be 17.6 grams. The weight of five adult F_1 males ranges from 21.0 to 29.8 grams, average 25.4. These fragmentary records indicate (and the general impression which one gets when handling the animals coincides with it) that the F_1 animals are nearly, though not quite, as large as the larger parental race. In vigor, productiveness, and longevity, however, the hybrids are far superior to either parent race.

The F_1 animals were crossed reciprocally with LITTLE's dilute brown race in order that segregation in size might be observed in relation to brown and dilution only without complication from pink-eye and short-ear which would continue to be recessive in whatever backcross animals received them from the F_1 parent. For the "d br" race, having been deliberately inbred, chiefly in brother-sister matings, since 1909, according to GREEN, should be completely homozygous for all genetic factors (except for possible new mutation), and it is free from pink-eye and short-ear, though homozygous for dilution and brown.

The backcross animals accordingly fall, as regards color, into four classes, (1) black, (2) dilute black (blue), (3) brown, and (4) dilute brown.

GATES (1926) thought that there was a tendency in a cross similar to this for the chromosomes to emerge from the cross in the same associations in which they entered it, so that in F_2 or in backcrosses a higher percentage was obtained of character combinations identical with those found in the parents than one would expect from chance alone. But GREEN did not observe any such tendency toward an association between chromosomes in the cross which he made between *M. bactrianus* (the supposed ancestor of the waltzing mouse) and the "d br" race of LITTLE. Further, a reëxamination of the data recorded by GATES raises a question as to the significance of the statistical deviations from expectation which he reported. It has seemed desirable, therefore, to reopen the question in connection with this investigation.

If GATES' association principle were valid for this cross, we should expect the parental combinations *intense black* and *dilute brown* to exceed in frequency the recombinations *dilute black* and *intense brown*. In the cross between F_1 females and "d br" males a population of 1001 animals recorded at weaning time (age 3 to 4 weeks) has been tabulated from the

records without selection. They consist of (1) black intense, 235; (2) black dilute, 268; (3) brown intense, 249; and (4) brown dilute, 249. The sum of the parental combinations (black intense and brown dilute) is 484, which is less than the sum of the recombinations (black dilute and brown intense), which equals 517. The probable error (expected chance deviation from equality in a population of this size) is 10.6. The observed deviation is 16.5, which is less than twice the P. E. and so not significant. It is also contrary in character (being minus rather than plus) to a deviation which might be due to persistent association of the chromosomes. The conclusion is warranted that in this cross, as in so many others which have been studied, the segregation of each chromosome pair occurs quite independently of other chromosome pairs, and continued association or dissociation of genes borne in different chromosomes is quite a matter of chance. We may therefore dismiss as groundless a criticism by CASTLE of GREEN's interpretation of his results based on the association principle outlined by GATES.

From the cross, F_1 ♀ × "d br" ♂, 1236 mice have been reared, weighed monthly or oftener between the ages of 4 and 6 months, and then chloroformed and measured as to body length and tail length by SUMNER's method, keeping the body under a uniform tension of 20 grams. In tables 1 and 2 the animals are classified as to sex, color, and maximum weight at or prior to six months of age. In tables 3 and 4 they are similarly classified as regards body length at six months of age.

The order of increasing size is the same for weight and for body length in both sexes. Blacks are smallest, then come blues, next browns, and last dilute browns. The only exception is found in body weight of dilute brown females, which are no heavier than intense browns, although as regards body length they are decidedly longer than intense browns. Body length is, as suggested by GREEN, a more reliable indicator of general body size than weight, for certain individuals in both the F_1 and the backcross populations have a tendency to become very fat after they have reached the age of 4 or 5 months, so that their weights displace the average upward. Body length, on the other hand, is apparently not affected by accumulation of fat, though it is clear that only individuals of large size become excessively fat. Body length is also a linear dimension, whereas increase in body weight depends upon increase in all correlated dimensions and thus produces greater variation. This is shown in the higher coefficients of variability for weight than for body length. For females the C. V. in weight is 10.8–12.0 and in body length 3.4–3.6; for males the C. V. in weight is 9.8–11.1, and in body length 2.9–3.8.

If we compare all black pigmented individuals (whether intense or dilute) with all brown pigmented ones of the same sex (whether intense

TABLE 1

Variation in weight of females of four different color classes from matings, ♀ F_1 × ♂ d br (Little).

COLOR	LOWER LIMITS OF WEIGHT CLASSES IN GRAMS																		NO.	MEAN	S.D.	C.V.
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32				
Black	2	1	3	15	16	28	35	23	13	11	6	5	1	0	1				160	21.46±.12	2.33±.08	10.8
Blue		2	2	11	17	24	21	35	23	16	11	9	0	0	4	0	1	1	177	22.32±.13	2.69±.09	12.0
Brown			4	6	13	22	30	20	21	11	17	11	2	5	2				164	22.61±.13	2.62±.09	11.5
Dilute Brown			4	5	10	28	24	28	21	12	12	10	4	2	1	2	1		164	22.61±.14	2.67±.10	11.8
All Blacks	Black and Blue combined																		337	21.91±.08	2.55±.05	Dif. means, .70±.14
All Browns	Brown and Dilute Brown combined																		328	22.61±.12	2.65±.07	
All Intense	Black and Brown combined																		324	22.04±.09	2.52±.06	Dif. means, .41±.13
All Dilute	Blue and Dilute Brown combined																		341	22.45±.10	2.67±.07	

TABLE 2

Variation in weight of males of four different color classes from matings, ♀ F_1 × ♂ d br (Little).

COLOR	LOWER LIMITS OF WEIGHT CLASSES IN GRAMS																							NO.	MEAN	S.D.	C.V.
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41				
Black	1	0	0	0	3	2	13	13	12	18	21	13	19	6	6	3	3	1	2						136	28.24±.16	2.77±.10	9.8
Blue		1	2	1	3	5	10	8	17	17	13	18	14	9	11	7	1	0	3	0	2	1	1		144	28.64±.17	3.04±.12	10.6
Brown					2	5	3	14	10	15	23	22	17	6	7	3	7	3	3	0	2				142	29.09±.18	3.24±.13	11.1
Dilute Brown						2	1	4	14	18	19	23	24	13	7	6	6	4	2	2	2	0	1	1	149	30.01±.17	3.18±.12	10.5
All Blacks	Black and Blue combined																								280	28.34±.13	3.40±.09	Dif. means,
All Browns	Brown and Dilute Brown combined																								291	29.56±.12	3.23±.09	1.22±.17
All Intense	Black and Brown Combined																								278	28.67±.12	3.16±.09	Dif. means,
All Dilute	Blue and Dilute Brown combined																								293	29.34±.13	3.52±.09	.67±.17

TABLE 3

Variation in body length in mm. of females of four color classes from matings, ♀ F_1 × ♂ d br (Little).

COLOR	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	104	NO.	MEAN	S.D.	C.V.	
Black		2	1	0	5	4	8	10	19	28	22	17	10	7	8	12	2	1	1	1			158	90.88± .17	3.22± .12	3.54	
Blue	1	0	0	0	3	4	5	10	12	15	24	23	23	15	18	4	9	6	2	1	1		176	92.13± .17	3.26± .12	3.53	
Brown				1	1	2	5	6	13	14	11	21	16	25	23	5	12	3	2	1	1		162	92.68± .17	3.21± .12	3.46	
Dilute Brown		1	0	1	1	3	1	8	10	12	11	21	14	19	21	15	10	4	4	2	0	1	159	93.00± .18	3.40± .12	3.65	
All Blacks									Black and Blue combined														334	91.51± .12	3.32± .08	Dif. means,	
All Browns									Brown and Dilute Brown combined														321	92.84± .12	3.26± .08	1.33± .17	
All Intense									Black and Brown combined														320	91.79± .12	2.72± .08	Dif. means,	
All Dilute									Blue and Dilute Blue combined														335	92.55± .12	3.36± .08	.74± .17	

TABLE 4

Variation in body-length in mm. of males of four color classes from matings, ♀ F_1 × ♂ d br (Little).

COLOR	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	NO.	MEAN	S.D.	C.V.
Black	1	0	0	1	0	1	1	3	2	7	6	14	9	20	13	15	15	14	6	4	2	1				135	95.52±.20	3.47±.14	3.63
Blue		2	0	0	1	1	2	2	2	5	7	9	10	12	18	20	22	12	7	4	1	2	0	2		141	95.91±.20	3.68±.14	3.83
Brown							3	1	1	1	7	4	8	15	14	23	19	21	13	4	2	1	2	1	1	141	96.96±.17	3.09±.12	3.18
Dilute Brown							1	0	1	1	0	3	5	9	13	16	26	23	14	12	11	6	2	1	2	146	98.46±.16	2.93±.11	2.97
All Blacks												Black and Blue combined														276	95.77±.14	3.50±.10	Dif. means,
All Browns												Brown and Dilute Brown combined														287	97.73±.10	3.11±.08	1.96±.17
All Intense												Black and Brown combined														276	96.26±.14	3.38±.10	Dif. means,
All Dilute												Blue and Dilute Brown combined														287	97.21±.14	3.57±.10	.95±.17

or dilute), we find the latter invariably longer-bodied and heavier in both sexes. For females, browns are 1.4 percent longer-bodied and 3.2 percent heavier; for males, browns are 2.04 percent longer-bodied and 4.3 percent heavier. See table 5.

If we compare the intense pigmented individuals (whether black or brown) with dilute pigmented (whether black or brown) of the same sex, we find the latter invariably larger and heavier. The difference is less in the

TABLE 5

Comparison of females with males as regards body length and weight in different color groups.

AV. BODY LENGTH	FEMALES	MALES	RATIO
All Blacks	91.55	95.77	100:104.6
All Browns	92.84	97.73	100:105.2
Ratio, Black:Brown	100:101.40	100:102.04	Mean=104.9
All Intense	91.79	96.26	100:104.8
All Dilute	92.55	97.21	100:105.0
Ratio, Intense:Dilute	100:100.82	100:100.98	Mean=104.9
Av. Weight			
All Blacks	21.91	28.34	100:129
All Browns	22.61	29.56	100:131
Ratio, Black:Brown	100:103.2	100:104.3	Mean=130
All Intense	22.04	28.67	100:130
All Dilute	22.45	29.34	100:130
Ratio, Intense:Dilute	100:101.9	100:102.3	Mean=130

case of dilution than it was in the case of brown, but still significant statistically in every case. See table 5. For females, the dilutes are 0.82 percent longer-bodied and 1.9 percent heavier; for males, the corresponding values are 0.98 percent and 2.3 percent.

The correlation between body-length and weight is high, being $0.65 \pm .01$ in the case of females and $0.66 \pm .01$ in the case of males. See tables 6 and 7. This supports the conclusion which one of us has based on a long series of investigations indicating that the genetic agencies which affect size are chiefly general rather than local in their action.

An interesting relation between the size characters of females and males is shown in table 5. Males are consistently about 5 percent longer-bodied than females of the same color group, but in weight they are about 30 percent heavier, the first difference being based on a linear measurement, the second on a mass or three-dimensional evaluation.

The differences between color groups are also greater relatively, as well as absolutely, in the case of males when compared with females. Brown makes an increase in body length (over black) of 1.40 percent in the case of females but of 2.04 percent in the case of males. Brown also increases

weight by 3.2 percent in the case of females, but by 4.3 percent in the case of males.

Dilution consistently has a lesser effect on size than brown, but its effect is regularly more pronounced in the case of males than of females. Thus dilution increases body length by .82 percent in females, but by .98 percent in males. It increases weight by 1.9 percent in females, but by 2.3 percent in males. The male mouse is a larger animal than the female. It grows beyond the stage at which growth is normally arrested in females, and in the final stages of growth the differential effect of genes borne in the chromosomes carrying brown and dilution is more strongly in evidence than before.

It remains to consider the question whether the differential effect on growth exercised by the brown and dilution chromosomes is due to the genes for brown and dilution respectively or to some gene or genes linked with them. FELDMAN (1935) has recently published data which indicate that in races of mice, whether of large or of small body size, browns in mixed litters are regularly larger than their black sibs.

In the cross described in this paper, we introduced from the *musculus* parent into the hybrids two closely linked recessive characters borne in the same chromosome, namely, dilution and short-ear. We have shown that in the backcross to "d br" males the individuals which are homozygous for dilution are larger than those which are heterozygous (the blacks and browns). Dilution (or something linked with it) must be regarded as an influence increasing size when homozygous. Short-ear does not reappear in this backcross, though present as a recessive in substantially all dilute individuals, since it would be transmitted to all individuals to which the F_1 parent transmitted dilution, unless a crossover occurred between short-ear and dilution.

But in a different backcross, to be described more fully in a subsequent paper, short-ear does make its appearance, and we are thus able to estimate its influence on size. When our F_1 females are backcrossed to the parental *musculus* race (pink-eyed short-eared dilute brown), the same four classes of offspring result as in the backcross already described, namely, black, blue, brown, and dilute brown; but in this case blues and dilute browns are short-eared. They are also regularly inferior in size to the blacks and browns respectively, which are long-eared. In other words, the tendency of homozygous dilution to *increase* body size, whether associated with black or with brown, is more than offset by a tendency of short-ear, when homozygous, to *decrease* body size.

But dilution and short-ear are closely linked with each other, with crossovers occurring about once in a thousand times. Any hypothetical size gene closely linked with one should also be closely linked with the other. Yet homozygous dilution increases size, and homozygous short-ear

TABLE 6

Correlation between body length and weight in 656 backcross females. r = .65 ± .01.

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	
15		1								1															2
16	1				1	1																			3
17		1	1	2	2			6																	12
18					5	6	3	7	6	4	3		1	1			1								37
19			1		1	3	9	4	12	5	6	8		3		1									53
20						1	3	9	14	25	10	16	7	5	7	2									99
21					1	1	3	3	9	15	18	23	13	9	8	3	2	1							109
22								2	8	9	20	16	15	16	10	6	3								105
23						1	1	1	2	5	3	10	11	12	16	9	5	3							79
24									2	3	4	4	6	6	13	3	4	3	1						49
25								1			3	3	4	5	7	8	7	4	2	2					46
26									1	1	1	1	5	7	6	3	5		2	2	1				35
27										1		1		1	1		1		2						7
28															1	1	2	2	1						7
29												2			1		2	1		1	1				8
30																	1							1	2
31														1				1	1						3
	1	2	2	2	10	13	19	33	54	69	68	84	62	66	70	36	33	15	9	5	2			1	656

TABLE 7

Correlation between body length and weight in 534 backcross males. r = .66 ± .01.

	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	
19					1																				1
20	2																								2
21							1																		1
22				1		1	1		2				1	1											7
23						4	1	2	1	2	1	1	1		1										14
24					1	1	1		4	2	6	5	3	2		2									25
25						1			1	8	7	4	5	2	4	2	1	1							36
26							1	2	2	3	5		9	6	4	6	1			1					40
27							1		3	1	7	7	11	12	14	4	6	1							67
28											4	2	10	9	14	15	11	6	1		1				73
29												3	7	11	12	17	11	9	1	4					75
30								1		2		1	6	10	14	9	13	8	4	2		1	1		72
31								1				1			2	9	7	5	3	3					31
32													2	2	2	6	5	1	6		4		1	1	30
33														1	2	4	2	2	4	1		1			17
34															1	2	6	1		1	2	1		1	15
35																	3	2	1	1					7
36									1					1		2	2	2	1	1					10
37																						1			1
38															1	1				1			1	1	5
39															1										1
40																1					1	1			3
41																			1						1
	2			1	2	7	6	6	13	19	30	22	55	57	72	80	68	38	22	15	8	5	3	3	534

decreases size, even in the presence of dilution. It is difficult to avoid the conclusion that these genes are, by virtue of their own physiological action, genes influencing body size. For if the influence on size were due to a third gene linked with short-ear and dilution, it should become operative when *either* short-ear or dilution become homozygous; but we find that size increases when dilution becomes homozygous but decreases when short-ear becomes homozygous, their action being qualitatively contrary.

SUMMARY

1. A cross was made between a pink-eyed dilute brown short-eared race of house mouse and an inbred strain of black-and-white Japanese waltzing mouse. Adult males of the parent races weighed, on the average, 26.2 and 17.6 grams, respectively. The F_1 hybrids were uniform black in color, and males, when adult, weighed about 25.4 grams.

2. A backcross was made between F_1 hybrid females and males of LITTLE's dilute brown race. The progeny fall into four color classes, black, blue, brown, and dilute brown, with no statistically significant differences between their respective frequencies. A backcross population of 665 females and 571 males was raised to the age of six months, each animal being weighed at monthly intervals and its body-length measured at six months of age.

3. In body-size, whether estimated by maximum weight or by body length, brown animals are larger than blacks, and dilute animals are larger than intense ones, the difference being greater in the case of brown than of dilution, but clearly significant in both.

4. Males are larger than females and show a greater influence on size of the brown and the dilute characters than females do.

5. Reasons are given for regarding the superior size of brown and dilute animals as due to the physiological action of the genes for those characters rather than of specific size genes borne in the same chromosomes.

6. The coefficient of correlation between adult weight and body length is, in the case of females, $.65 \pm .01$, and of males, $.66 \pm .01$.

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